ON THE SURVIVAL AND TRANSPLANTABILITY OF ADULT MAMMALIAN TISSUE IN SIMPLE PLASMA.*

By ALBERT J. WALTON, M.S.

(*From the Bacteriological Laboratory of the London Hospital, London.*)

PLATES 22 TO 26.

This communication deals with the characteristics of the growth of adult mammalian tissues after transference to fresh medium. In all cases the medium was simple plasma, and the tissues were therefore not stimulated by the addition of tissue extract.

In 1911 Carrel[1] published the results of his experiments on the continued growth of thyroid tissue in simple plasma. At that time he stated that he had not obtained a third generation of cells. Later he and his assistant Ebeling published several papers[2] in which they describe growth persisting for over a year, but in the majority of these cases the subcultures were made in a medium of plasma and tissue extract so that growth was stimulated.[3] Even in such cases Carrel informs me that he has noticed a tendency for the cultures of adult tissues cultivated in media which contain no tissue extract to die; and in a later paper[4] he states that even with embryonic tissue the growth after several passages in normal plasma alone becomes progressively reduced and often stops altogether.

It is necessary that the characteristics of the growth of unstimulated adult tissue be considered before any conclusions can be made as to the effects of various substances upon the rate of growth. The present experiments are therefore concerned only with the

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[1] Carrel, A., and Burrows, M. T., *Jour. Exper. Med.*, 1911, xiii, 416.

[2] Carrel, *idem*, 1912, xv, 516. Ebeling, A. H., *idem*, 1913, xvii, 273.

[3] Carrel, *idem*, 1913, xvii, 14.

[4] Carrel, *idem*, 1913, xviii, 287.

growth of tissue taken from the adult rabbit in the unaltered plasma of the same animal.

The technique used in the preparation of the primary cultures is that of Carrel,[5] which I have described fully elsewhere.[6] In preparing the subcultures, or transferences, the piece of growing tissue was cut out of the plasmatic medium with a cataract knife and transferred to a bowl of sterile Ringer's solution, where it was washed for a few seconds. A drop of plasma was placed on a fresh sterile cover-slip, the piece of tissue transferred to it, and the plasma spread evenly with a cataract knife. After a few seconds coagulation of the plasma had taken place and the cover-slip was then inverted over a cell slide and sealed in position with molten paraffin. The preparation was then placed in the incubator. Throughout the experiments the most careful aseptic precautions were used, as there is considerable risk of infection. The various steps including the washing in Ringer's fluid were carried out at room temperature, the plasma alone being kept on ice to prevent coagulation. In most cases the whole piece of tissue was transferred, but if growth was well marked it was divided into two, three, or four pieces, each of which was transferred to the new plasmatic medium.

The total number of cultures made in the present investigation was 147. Of these 115 showed growth in the first culture, the remainder probably having died owing to some technical error, as they were mostly isolated specimens in a series of six made from the same tissue and under identical conditions. The characters of the growth of the subsequent subcultures, which varied with the nature of the tissue used, will be descibed below.

Thyroid.—Of this tissue thirteen cultures were made, being taken in groups of six, three, and four from different animals on different dates. Of these thirteen cultures only nine grew, but these when subcultured continued to grow until the third or fourth generation. The specimens from the first animal grew well until the tenth generation, but after this they died suddenly. This was probably in part due to the fact that the plasma of a hare was accidentally

[5] Carrel, *Jour. Exper. Med.*, 1912, xv, 516.
[6] Walton, A. J., *Jour. Path. and Bacteriol.*, 1914 (in press).

used instead of that of a rabbit. Up to the time of death the cultures had lived for a period of forty-two days.

The tissues of this animal showed after the first transference a marked increase of growth, so that whereas in the first culture growth was present only after an interval of two days, in the second and third subcultures there was usually well marked growth after twenty-four hours. Branching, irregular cells were seen projecting from the edge of the tissue (figure 2), and, if any of the old plasma had been transferred with the tissue, the cells soon passed beyond this into the new medium (figures 1 and 2). Here they rapidly grew and formed an irregular network of cells (figure 3). After a further interval of twenty-four hours the network had become sufficiently dense to form a mass of new tissue resembling the original mass that had been transferred (figure 4). If this mass showed marked increase the tissue was divided before transference. In this way one piece of tissue from this animal was subdivided several times, so that after an interval of twenty-one days there were at the fifth subculture nine pieces of tissue each of the same size as the original piece. At this time growth was such that at the end of the second or third day the ring of new growth was equal in width to the diameter of the original piece of tissue. If a piece of rapidly growing tissue was divided into two it was noticed that the growth was delayed on the cut edge, apparently from the trauma, so that when the cells from the uncut edges were forming a halo of branching cells spreading out into the plasma there were only a few cells projecting from the cut edge. These cells, however, grew rapidly and after the second or third day nearly equalled in width the cells growing from the uncut edge. In the case of the other animals, where growth of the thyroid was not prolonged beyond the third or fourth generation, the rapidity of growth decreased markedly after the first transference. In the first subculture growth was more rapid than in the primary culture, as in the case described above. With the second subculture growth occurred more slowly, being no more rapid than in the case of the first culture, or perhaps even less rapid. At the third transference the cells grew slowly, and if a culture was left for five or six days without transference they became rounded instead of branching, and granular in appearance. In a

short time vacuolation of the plasma occurred and granular remains of cells were found floating free in the liquid of the vacuole. If, however, transference was made on the second day a few cells of short life might occasionally be seen.

The difference in the rate and extent of the growth of the tissue of the first and subsequent animals was possibly due to difference in the age of the rabbits used. As the animals were purchased and not bred in the laboratory, it was impossible to estimate their age accurately, but it was noticed that the first animal used in this series was small and apparently young. This question will, however, be considered in a later communication.

Spleen.—Of this tissue sixty-eight cultures were made from twelve different animals, being taken in ten groups of six each, two groups of three and one of two. In all cases growth was present in the first culture, the growing cells being mainly round and wandering cells. Transference was made at periods varying from three to six days; if left later than this the percentage of growths was definitely decreased. After the first transference thirty-four only showed signs of growth, that is, 50 per cent. Of these all showed marked increase in the rate of growth and a very definite change in the nature of the cells. Thus after twenty-four hours a large number of round cells were seen, but in addition to these a few spindle cells were seen projecting from the edge of the tissue. These increased so that by the third or fourth day the tissue was surrounded by a ring of radiating cells as wide as the piece of tissue (figure 5). The cells appear to be of the connective tissue type. The pieces of tissue were again transferred on the second or third day, and in five the growth was so extensive that the piece of tissue was divided into two parts. Of the forty-three resulting cultures of the third generation eighteen alone showed growth. In these the growth was almost wholly of long radiating cells of spindle shape and apparently connective tissue. The growth, however, was not nearly so extensive as in the second generation, and in several, at the end of two or three days, only a few radiating cells were seen passing out into the new medium. In many of these cells globules resembling fat in appearance were seen, and after a short time the cells began to degenerate. The protoplasm became irregular and ill defined, so

that on staining it was not sharply differentiated from the surrounding plasma. The nuclei stained poorly and were also not sharply defined. In the fourth generation only four pieces of tissue showed evidence of growth, and in all cases this was very slight. A few growing cells could be seen on the first or even on the second day, but after this they rapidly disappeared, being converted into a granular mass.

Testicle.—Of this tissue eighteen cultures were made, being taken in groups of six from three different animals. Growth occurred in fourteen in the first culture. The growth was well marked and showed the usual characters of testicular growth. On the third day growth was sufficiently far advanced for subculture. In the second generation the cells grew well and as usual more rapidly and extensively than in the first culture. The cells were of the spindle type, but more closely resembled those of the first culture than in the case of the spleen. Although these cells are spindle cells and are possibly connective tissue, yet they contain more protoplasm and are less elongated than the cells occurring in subcultures of the spleen, so that it is generally possible even in the third and fourth generations to distinguish one tissue from another by the appearance and arrangement of the cells, although in no case is there any reproduction of the normal histological picture of that viscus. For the third culture growth had become so marked that seven pieces were divided into two, resulting in sixteen cultures of the third generation. Of these only eight grew, but they showed extensive and rapid outgrowth of cells (figures 6 to 9). The onset of growth was, however, not so rapid as in the second generation, there being only a small halo of cells after forty-eight hours. After this growth was rapid, and in a further twenty-four hours long projecting masses of cells were seen passing into the surrounding plasma. By the fourth day the cells formed a ring wider than the original piece of tissue (figure 9). Four of the specimens were killed and stained for photographs, and the other four were subcultured. Only one of the latter showed growth, and that to a slight extent, so that after two days a few cells were seen projecting from the edge. These had slightly increased on the third day, but on the fourth the outlines were ill defined and the protoplasm was granular,

while a brown granular deposit had commenced to form in the plasma around the cells.

Kidney.—Of this tissue thirty-two cultures were made from five different animals, being taken in three groups of six and two groups of eight. Of these only fifteen showed growth in the first culture. As described in a previous communication, the growing cells were almost wholly epithelial in type, forming large plate-like masses of cuboidal cells. Transference was carried out on the third to the fifth day, according to the extent of the growth in the first culture. In the second generation eleven showed growth. This was more rapid than in the first culture and the cells preserved the characteristics of the first culture, being mainly of the epithelial type. The presence of cells of the connective tissue type was, however, rather more marked than in the first culture. There was again a tendency to early vacuolation of the plasma (figure 10). Many of the cells showed evidence of mitosis in the nuclei (figures 11 and 12), but owing to the difficulties of staining in bulk the figures were not as clear as one would desire, although they were more marked in this type of cell than in the spindle-celled connective tissue type. The eleven growing tissues were subcultured, but growth took place only in three, in all of which it was slow and more definitely of the connective tissue type than in the second generation. In no case was growth obtained in the fourth generation.

Liver.—Of this tissue fourteen cultures were made from two animals in groups of eight and six. In nine of these there was active growth. This commenced on the second day and transference was carried out on the third day. In the second generation evidence of commencing growth was seen in three specimens after twenty-four hours. In the other six there was no evidence of growth. Although in the first generation there were a large number of cells which were more or less rounded and which contained a relatively large amount of protoplasm, yet in the second generation the cells were all radiating and of the connective tissue type. This was visible even on the second day (figure 13). The cells grew well and rapidly spread out into the surrounding plasma, so that transference was carried out on the third day. In one case

only in the three specimens of the third generation was any growth observed, and this was only slight. A few radiating cells were seen on the second day, but after a short time these became granular and a brown deposit occurred in the surrounding plasma. The outline of the cells became indefinite and difficult to distinguish from the surrounding plasma, the plasma became vacuolated, the granular deposit increased, and soon all signs of growth ceased.

CONCLUSIONS.

1. Growth of adult mammalian tissue can be prolonged by transference to fresh medium.

2. In a few cases this growth can be continued for ten or eleven generations up to a period of forty days.

3. In the majority of cases growth ceases after three or four generations.

4. After the first transference growth is increased, but in subsequent generations it gradually diminishes and ultimately ceases altogether.

EXPLANATION OF PLATES.

PLATE 22.

Tissue = adult rabbit thyroid; medium = rabbit plasma; culture = fifth subculture.

FIG. 1. Standard. Fixed immediately after coagulation. No growth. 35/1.

FIG. 2. After forty-eight hours. Well marked commencing growth. 35/1.

FIG. 3. After three days. Cells commencing to form a network. 35/1.

FIG. 4. After four days. Well marked network of cells. 35/1.

PLATE 23.

Tissue = adult rabbit spleen; medium = autogenous rabbit plasma; culture = second.

FIG. 5. Fourth day. Marked growth of radiating spindle cells. 35/1.

PLATE 24.

FIG. 6. Tissue = adult rabbit testicle; medium = autogenous rabbit plasma; culture = third. Standard. Fixed one quarter of an hour after coagulation. 35/1.

FIG. 7. After forty-eight hours. 35/1.

FIG. 8. After three days. 35/1.

FIG. 9. After four days. 35/1.

PLATE 25.

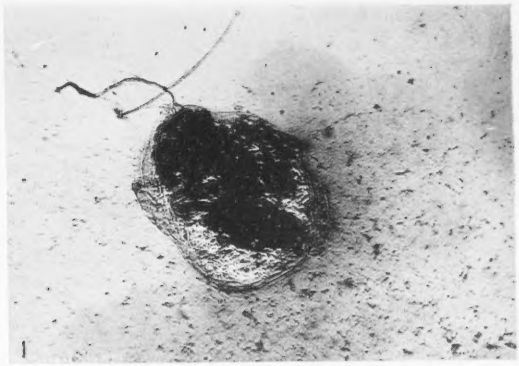
FIG. 10. Tissue = adult rabbit kidney; medium = rabbit plasma; culture = second. After two days. Growth of masses of epithelial cells. Vacuolation of plasma. No growth in vacuole. 35/1.

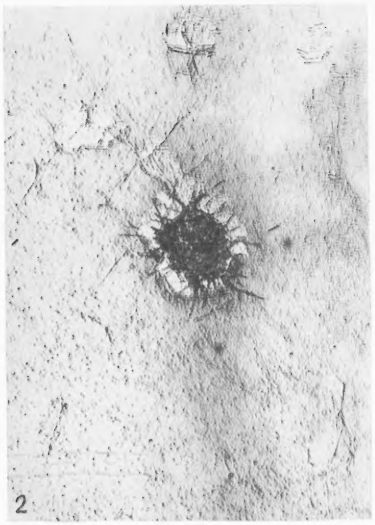
FIG. 11. Same specimen as figure 10. Enlarged to show the appearance of the cells.

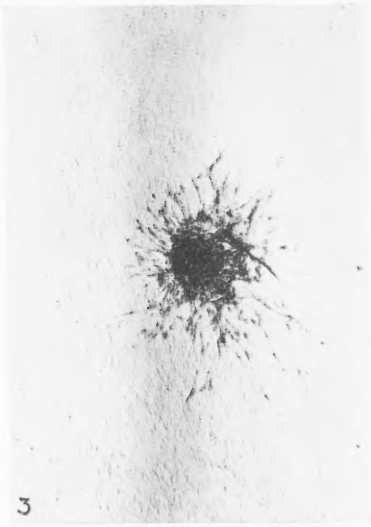
PLATE 26.

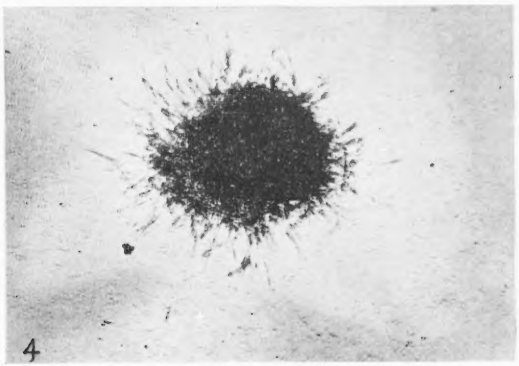
FIG. 12. Tissue = adult rabbit kidney; medium = rabbit plasma; culture = second. Same specimen as figure 10. Enlargement of the growing edge, showing various stages of mitosis in the nuclei.

FIG. 13. Tissue = adult rabbit liver; medium = rabbit plasma; culture = second. After forty-eight hours. Growth of radiating spindle cells.

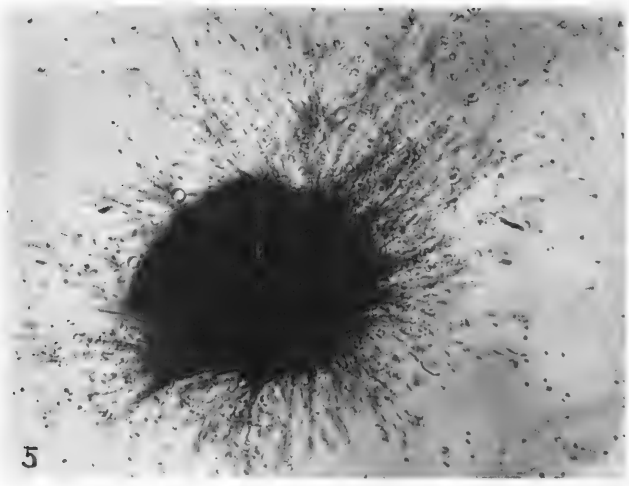








(Walton: Adult Mammalian Tissue in Simple Plasma.)



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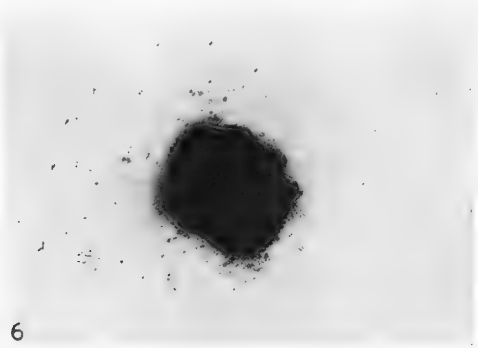

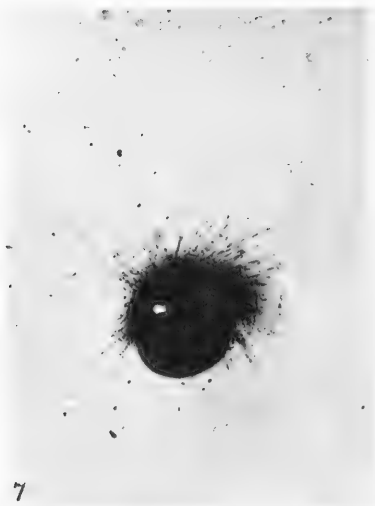

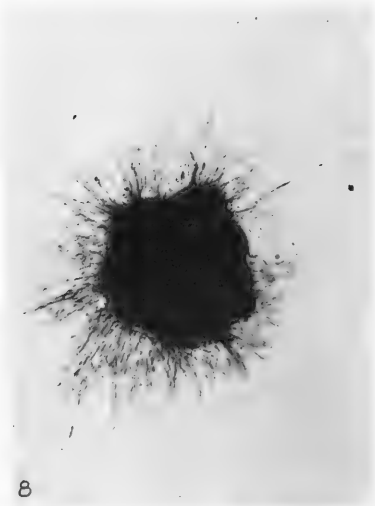

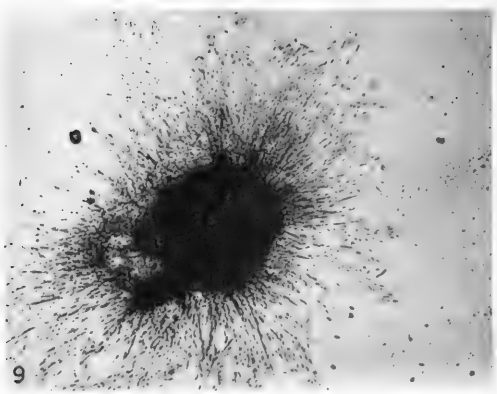

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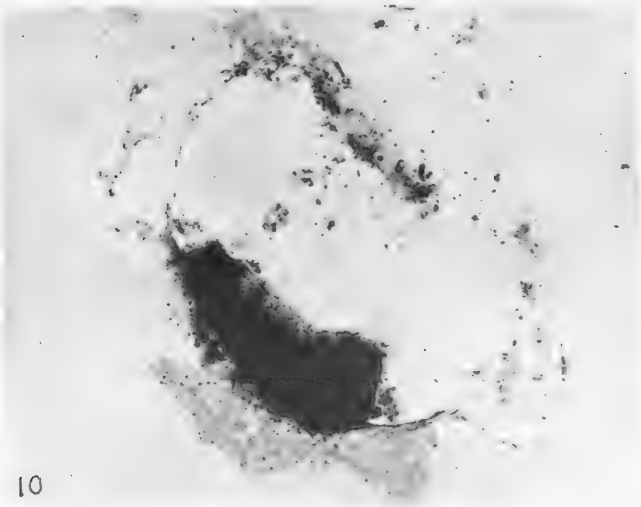

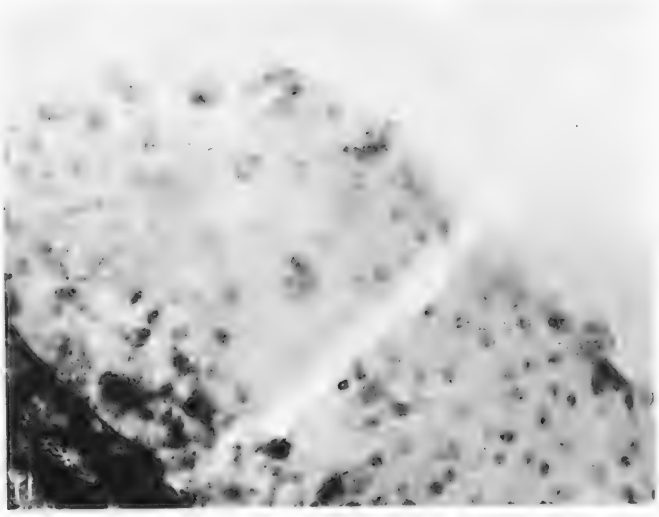

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THE BEHAVIOR OF ELASTIC TISSUE IN THE POST-FETAL OCCLUSION AND OBLITERATION OF THE DUCTUS ARTERIOSUS (BOTALLI) IN SUS SCROFA.*

By J. PARSONS SCHAEFFER, M.D.

(From the Anatomical Laboratory of the Department of Medicine, Yale University, New Haven.)

Plates 27 to 32.

INTRODUCTION.

Much has been written on the morbid anatomy of blood vessels, including especially such pathological conditions as arteriosclerosis, endarteritis obliterans, thrombo-angiitis obliterans, and arteritis elastica, and reference has occasionally been made to the behavior of elastic tissue in the pathological condition under discussion. Likewise some experimental work has been done in an attempt to simulate diseased conditions in blood vessels, and incidentally some light has been thrown on the nature of the proliferation of elastic tissue in these states. Nevertheless, further work and experimentation are needed in order to elucidate the true conditions involved in the genesis and the proliferation of elastic tissue in postembryonic material.

Most of our knowledge concerning the proliferation of elastic tissue in postembryonic material is based on the study of pathological tissues (Langhans, Baumgarten, Thoma, Wendeler, Dmitrijeff, Hilbert, Jores, Buerger, and others).

The writer has already reported[1] on the occlusion and ultimate obliteration as such of the ductus arteriosus (Botalli) in man.[2] In

* Received for publication, October 16, 1913.

[1] Read before the Association of American Anatomists, Cleveland, 1912.

[2] The studies on the occlusion and obliteration of the ductus arteriosus in man were carried on in the Pathological Laboratories of Bellevue Hospital, New York, and in the Anatomical Laboratory of the Yale University Medical School. I wish to express my gratitude to Dr. Charles Norris, Pathologist to Bellevue Hospital, for many courtesies and for the privilege of working in his laboratory and in the Bellevue morgue. I am also indebted to the authorities of the New York Foundling Hospital for the opportunity of working in the hospital morgue.

this communication reference was made to the behavior of the elastic tissue contained in the ductus during the stages of the retrogressive processes, and to the difficulty even in this material of ruling out disease absolutely in all cases. The material was from young infants, yet some of it gave evidence of an arteritis, probably of syphilitic origin. However, a comparative study may show that elastic tissue proliferation in postnatal tissues is similar in both pathologic and healthy proliferating processes.

MATERIAL.

In order to study the nature of the increase of elastic tissue in material where disease is a negligible factor, the postnatal ductus arteriosus of *Sus scrofa* was selected. Here one finds an active formation of new elastic tissue in the occlusion of the lumen of the ductus, yet the process of occlusion can not truly be classified as pathological. There is no evidence of an inflammatory condition, notwithstanding that there is an active proliferation of the tissues composing the intima.

Prenatal material of different stages was obtained from a local slaughter house. The postnatal material studied was mostly from two litters of pigs. The live pigs were delivered at the laboratory at requested intervals, and in this way they were left with their mothers and under normal environment until wanted for study.

PRENATAL DUCTUS ARTERIOSUS.

A study of embryonic material was deemed essential as an aid in the interpretation of conditions found in postnatal material. No detailed study of the histogenesis of elastic tissue in the ductus arteriosus of *Sus scrofa* was undertaken. However, a brief study of very early material was not in accord with Ranvier's statement, that elastic fibers are formed by a coalescence of elastic granules. Early individual elastic fibrils appear distinctly granular or even as rows of granules, and at first thought one is tempted to interpret the apparent row of elastic granules as the stage immediately preceding the elastic fibril. The granular condition appears more evident in certain planes of section, while in others the granule is

more or less lost and the elastic fibril appears more like a continuous line. The direction of the elastic fibers in the ductus is more or less complicated at the aortic and pulmonary ends owing to the union of the ductus with large vessels. Mall's suggestion that "the granules are optical sections of fibrils of elastic tissue" seems to the writer the correct interpretation of the granular appearance.

The material studied of the early stages indicates that elastic fibrils in the ductus arteriosus of *Sus scrofa* are directly differentiated, and is in accord with the conclusions of Mall and Spalteholz. There seems to be a fusion of elastic fibrils in the production of the heavier elastic lamellæ.

In a study on the histogenesis of elastic tissue in arteries Mall concludes "that the exoplasm of the connective tissue syncytium forming their walls differentiates into two kinds of fibrils, which give rise to the white fibrous and elastic tissues, respectively. In other words, one cell gives rise to both tissues." I, too, find in the occlusion of the lumen of the ductus arteriosus in *Sus scrofa* that besides a great proliferation of elastic fibers from preëxisting elastic tissue there is also a production of elastic fibrils from non-elastic elements, apparently from connective tissue cells.

Just what the true nature of the process is by which white fibrous and elastic fibers are formed from the exoplasm of connective tissue cells and how they come to occupy an intercellular position is yet in a sense controversial ground. Ferguson, for example, in a recent contribution on some experimental work, after dismissing the theories of direct transformation by elongation of mesenchymal cells into connective tissue fibers, and the origin of connective tissue fibers from the ground substance between the cells, apparently adheres to some form of intracellular theory, but he considers that "none of the theories thus far advanced fully meet all of the observed conditions." It is, however, not the purpose of this paper to discuss at length the histogenesis of elastic tissue in embryonic material. Suffice it to say that the material studied gave evidence, as is generally held, that both elastic and white fibrous tissue fibers are laid down in the outlying portion of the protoplasm of the early connective tissue cell.

During the latter half of fetal life the ductus arteriosus of *Sus*

scrofa is found to have an exceptionally well developed inner elastic membrane. There is, however, no sharp demarcation between media and adventitia. The outer elastic membrane is for the most part but faintly differentiated, and the media at most points merges imperceptibly with the adventitia. Cross-sections of the midportion of the ductus best show the strong inner elastic membrane, made up in many places of a single lamina with sharp definition. However, on one side of the ductus the inner elastic membrane is frequently made up of two or three distinct laminæ. Wherever the bi- or trilaminar condition of the inner elastic membrane prevails the subendothelial layer is thicker than elsewhere (figure 4).

Just what the multilaminar condition of the inner elastic membrane means is problematical. It would, however, seem that it is a compensatory increase in tissues to make up for a possible greater strain at these points of the ductus during the passage of blood through it. Thoma, for example, found a special thickening of the intima to take place in older vessels which had become wider under continued blood pressure. In this way he claimed that the normal lumen was more or less maintained. The observation has also been made[3] that in the blood vessels of the cranial cavity the inner elastic membrane is apt to split up into two or more layers whenever there is need for counteracting an excessive amount of pressure, as, for example, at points where branches are given off, where two vessels come together, and on the concave side of a bent vessel.

POSTNATAL DUCTUS ARTERIOSUS.

The postnatal behavior of the elastic tissue in the ductus arteriosus of *Sus scrofa* is somewhat complicated and irregular. This is in part due to the fact that we are dealing with a vessel undergoing regression, involving first an occlusion of its lumen and subsequently its obliteration as such. In the occlusion of the lumen similar conditions do not always obtain in the several segments of the postfetal ductus, neither is the occlusion necessarily uniform in different individuals.

After birth the ductus soon presents in a considerable portion of its course a markedly irregular and contracted lumen (figures 6

[3] Quain, J., Anatomy, 11th edition, London, 1912, ii, pt. 1.

and 9). This is usually most marked somewhere along its midportion. The contraction varies in degree, leading from partial (figure 9) to almost complete occlusion (figure 13) of the lumen of the vessel. In other portions of the ductus, especially in the terminal thirds, the contraction is, as a rule, not so marked, as may also be the case throughout the ductus in other individuals. It should here be noted that even before birth there is often some irregularity of the lumen.

Where the contraction is most marked one finds early in the neonatal ductus a slight proliferation and subsequent regression of the endothelium and a breaking up of the inner elastic membrane into several layers. With these changes there is an apparent fusion of opposed endothelial surfaces. Where the contraction only partially occludes the lumen the marked hyperplasia of the intimal tissues completes the process. In some instances there are portions of the ductus where a questionable thrombosis with subsequent organization plays a part (figures 5, 11, and 16).

One of the first changes in the elastic tissue of the postfetal ductus arteriosus of *Sus scrofa* is a hypertrophy of the inner elastic membrane throughout the vessel. This takes place regardless of the degree of contraction and irregularity of the lumen. There is, apparently, also a slight hypertrophy of the elastic fibers of the media and adventitia, but this is insignificant in comparison with the thickening of the inner elastic lamina. Soon one encounters the characteristic delamination of the thickened inner membrane, *i. e.*, a number of new laminæ are formed from the old membrane. The new laminæ run more or less parallel with the mother lamina, both of which now follow the postfetal irregularities of the lumen of the ductus. The formation of new elastic laminæ is not wholly at the expense of the old inner elastic membrane, for, aside from the formation of new lamellæ from the latter, there is also a simultaneous hypertrophic process at work.

The production of new elastic tissue from the internal elastic membrane begins soon after birth, as does also the proliferation of the other tissues composing the intima. A study of the ductus of a fourteen day pig showed that the inner elastic membrane at the pulmonary end had already hypertrophied and split into a large

number of distinct lamellæ (figure 9). In the midportion of the ductus the internal elastic membrane was composed of from three to four lamellæ, while at the aortic end it was bilaminar in character. Here and there fine elastic fibrils were detected amongst the cells beneath the endothelium. The new elastic lamellæ were not everywhere entirely independent, but were either still a part of the old inner membrane or attached to neighboring new lamellæ. The greatest proliferation of new elastic lamellæ from the internal elastic membrane was in regions where the contraction of the lumen was least marked. Connective tissue cells were lodged in some of the meshes of the multilaminar internal elastic membrane.

At times the formation of new elastic lamellæ is marked, and it is obvious that elastic tissue plays an important part in the occlusion of the lumen of the ductus. In figures 10 and 15 are represented cross-sections of ductus arteriosus from eight weeks old pigs. Here heavy new elastic lamellæ, doubtless a product of the inner membrane, make up a large portion of the occluding mass of the lumen of the ductus. In many places the new lamellæ are independent, but by following the different stages in their production one is led to see that they are a product of preëxisting elastic tissue. Aside from the heavier elastic fibers there is also present in the sections a finer network of elastic tissue to which reference will be made subsequently (figures 8 and 10).

Figure 16 illustrates the conditions found in the aortic end of the ductus arteriosus of a six weeks old pig. It will be noted that the inner elastic membrane is more or less split into lamellæ. The lumen of the ductus is entirely occluded by new tissue. A number of large elastic lamellæ are found in the occluding mass. However, there is a greater abundance of elastic tissue irregularly disposed. It is indeed difficult in such cases to decide between the fibers formed from preëxisting elastic tissue and those of non-elastic origin.

The marked breaking up of the thickened inner elastic membrane into a number of elastic lamellæ and fibers in the occlusion of the ductus is, according to the material studied, much more characteristic of the pig than of man. In the latter one often finds a marked thickening of the inner elastic membrane and the production of a fine network of elastic tissue which ramifies the hyperplastic sub-

endothelial layer and with the latter projects cushion-like at various portions of the ductus into its lumen. Again, one finds, in man, specimens in which there is a definite elastic lamina in the position of the internal elastic membrane and another lamina next the lumen of the partially occluded ductus. Between the two heavier elastic laminæ is found a finer and more or less homogeneous mass of elastic tissue. One does not often find in man so extensive a delamination of the thickened internal elastic membrane as in *Sus scrofa* (figures 1, 2, and 3).

As stated before, in the occlusion of the lumen of the ductus arteriosus in *Sus scrofa* elastic tissue plays an important part, and this is largely due to the marked proliferation from the internal elastic membrane. However, aside from the formation of new elastic fibers from preëxisting elastic tissue, there is also an independent production of elastic fibers, that is, from non-elastic tissue. The latter process is especially active where the contraction of the ductus is less marked and where a rather large lumen is left to be filled in by intimal hyperplasia or otherwise.

Unfortunately the part of this investigation bearing on the non-elastic origin of elastic tissue in postfetal material, and the behavior of elastic tissue in the organization of thrombi is yet incomplete. Experimental work bearing further on these problems is now in progress, and the results will be reported subsequently. Reference will here be made to preliminary work only.

In some recent double ligations of the left common carotid artery in very young pigs there was noticed after an interval of from five to eight days a slight thickening of the intima. The latter appeared unusually cellular, and in places a rather faint but positive network of granular lines was brought out by orcein stains. Closer examination showed that in the meshes of the very delicate network, apparently elastic in character, were connective tissue cells with rather large round or oval nuclei with little protoplasm surrounding the nucleus. The faintly outlined, fine granular fibers appeared to be at the edge of the cell limit. In specimens where greater intervals were allowed between the operation and the study of the ligated vessel the network of elastic fibers surrounding cells of the subendothelial layer was more evident. Notwithstanding the presence of

delicate elastic fibers in the slightly thickened intima, the inner elastic membrane was normal and unbroken,—a marked contrast with the condition found in the neonatal ductus arteriosus of *Sus scrofa*. However, as stated previously, these observations are merely preliminary, and it will be necessary to study ligated vessels at repeated intervals up to obliteration and organization of the filled in lumen before any conclusions can be drawn on the behavior of elastic tissue under such conditions.

The preliminary study seems, however, to indicate that in ligations of this character there is early a formation of delicate elastic fibrils in some manner from the cells in the subendothelial layer. Jores believes that the formation of new elastic tissue within the hyperplastic intima in endarteritis is directly due to the activity of the protoplasm of intimal cells.

The conditions that obtain early in the postfetal ductus arteriosus of *Sus scrofa* make the proliferation of elastic tissue more difficult of interpretation. While one does encounter at places very fine elastic fibers amongst the cells of the subendothelial layer, there is also the marked production of elastic lamellæ from the inner membrane previously referred to. It is, therefore, difficult to distinguish between elastic fibers derived from preëxisting elastic tissue and those of other origin (figures 7, 8, and 10).

One notices, however, that the finer, irregularly disposed fibrils take the specific stains less deeply than do the lamellæ which are obviously a product of the inner elastic membrane. Furthermore the finer fibrils bear more intimate relations to the protoplasm of the connective tissue cells, *i. e.*, they inmesh the cells. The fine elastic fibers soon become thicker and by migrating become mingled with other fibers. Their real identity then becomes even more vague. Again, there is a similarity between the finer elastic fibers contained in the occluding mass of the lumen of the ductus arteriosus of *Sus scrofa* and those found in the thickened subendothelial stratum of the ligated common carotid arteries previously referred to. It would, therefore, seem that in the occlusion of the ductus there is early a production of elastic fibrils from the protoplasm of the cells contained in the subendothelial layer, as well as

the more extensive production of new elastic fibers by a proliferation of the thickened inner elastic membrane.

As the process of occlusion of the lumen of the ductus advances the new elastic tissue becomes more and more abundant and additional facts are brought out in confirmation of a genesis of elastic fibrils from non-elastic elements. Where the lumen of the ductus is markedly contracted little proliferation of the tissues of the intima is needed, as stated before, to bring about occlusion (figure 13). On the other hand, one encounters ductus and segments of ductus where the contraction is much less, and in order that occlusion may be effected a proliferation of intimal tissues or a thrombosis with subsequent organization, or both, is necessary.

The behavior and disposition of the elastic tissue in the ductus and parts of ductus where a rather large lumen remains to be filled in, is best studied in pigs from the fourth to the ninth week. Then one finds a considerable amount of elastic tissue in the occluding mass, and it is obvious that elastic tissue proliferation plays an important part in the occluding process. A reference to figures 10 and 15 indicates the previously referred to thickening and delamination of the inner elastic membrane. The new elastic laminæ with other proliferated intimal elements have wandered into and occluded the lumen of the vessel. Besides the heavier elastic lamellæ which in part run more or less parallel to the folded old inner elastic membrane, there are present numerous finer elastic fibers, arranged irregularly and somewhat in a network. Often these finer fibers have no connection with the larger and more regular elastic lamellæ and stand independently. Of course, as the process advances there is obviously a fusion of the finer fibers and they become more and more intermingled with and like the coarser fibers. If one is, however, fortunate enough to get the material at opportune stages it is obvious that not all the elastic tissue found in the lumen of the ductus is formed from preëxisting elastic tissue.

Again, one finds portions of some ductus in which the occluding process is complicated by the presence of blood amongst the tissues making up the occluding mass, and one questions whether in such portions of the ductus the process is merely a hyperplasia of the intimal tissues. Just what the correct interpretation of the condi-

tion is, is difficult to say. Is there for a time a seeping of blood through the loose occluding mass or is the latter a thrombus undergoing organization? As the occluding mass becomes denser, according to my material, the blood is mostly collected into definite channels which for a time take on the form of definite vessels that run parallel to the original lumen of the ductus. These channels are variable in number and size in different portions of the ductus, and there is no constancy in their disposition, size, or number in different individuals. This is, of course, to be expected owing to the different conditions that usually obtain in different portions of the neonatal ductus. The channels are usually more or less cylindrical, have thin but definite walls, and in many instances a well formed inner elastic membrane, the latter obviously not a product of preformed elastic tissue. Are these channels which contain blood temporary blood vessels formed within a vessel undergoing regression and occlusion, or do they represent canalizations of a thrombus? The fate of these channels and the behavior of the elastic tissue adds some light on these questions.

The vessels or channels frequently found in the occluding mass are for the most part short lived (figures 11 and 12). As the occluding mass of the lumen of the ductus becomes denser there is also apparently a proliferation of the tissues composing the walls of the channels. Ultimately these processes cause the channels to suffer the same fate as the mother ductus, *i. e.*, occlusion and obliteration. In place of the channels nests of cells are subsequently found (figure 12), and when specific stains are used definite masses of elastic tissue are found here and there in the mass occluding the lumen, obviously the site of the former blood channels (figure 17).

On the whole, the channels do not behave like canalizations of thrombi observed in pathological material. Whether the ultimate fate of canalized channels in thrombi is, in some instances, similar to the blood channels frequently found in the mass occluding the lumen of the ductus arteriosus of *Sus scrofa* I have been unable to observe. Hematoxylin and eosin-stained sections of such segments of ductus, as above referred to, look somewhat like organizing thrombi (figure 11). In the latter there is some round cell infiltration and a considerable amount of blood diffused through the mass.

In later specimens the blood is found for the most part in definite channels which are found in the mass. Fibroblastic cells, which probably took origin from the proliferated endothelial lining or from the cells in the hyperplastic subendothelial stratum, are also present in the mass. Elastic tissue is present in abundance. Here and there, especially around the blood channels in the mass, are found elements that look and behave like unstriped muscle cells. Modified endothelial cells are also encountered. However, only by staining sections from the same segment of the ductus with specific stains is the richness of elastic tissue in the occluding mass and the marked delamination of the thickened internal elastic membrane brought out. The disposition of the elastic tissue was previously referred to (figures 11 and 15).

Buerger, who recently made a study of blood vessels from cases of presenile gangrene, considers the obliterating process found in such cases a thrombo-angiitis obliterans. He believes that the behavior of the elastic tissue distinguishes an obliterating arteriosclerotic process (arteritis obliterans) from a thrombo-angiitis obliterans. In the former there is, of course, among other things a marked hyperplasia of the intimal tissues; in the latter primarily an organization of a thrombus. Buerger finds in his cases of thrombo-angiitis obliterans, "either a total absence of elastic fibers, or when such are present, a growth of fibers around the larger canalizing vessels, particularly about those which are thick walled and old." I recently had the opportunity of examining a few small vessels taken from a case of presenile gangrene and found in the sections studied that the organizing thrombi contained no elastic tissue. The inner elastic membrane was sharply demarcated and not delaminated, as in the neonatal ductus of the pig. However, the material was too limited to permit one to draw conclusions in this connection. Jores in some experimental work apparently demonstrated elastic fibers in organizing thrombi. As stated previously, the work on the behavior of elastic tissue in ligations, organization of thrombi, etc., is as yet incomplete, as is also the work on the postfetal occlusion of blood vessels. Experimental studies bearing further on these problems are now in progress, the results of which will be reported subsequently.

ADULT DUCTUS ARTERIOSUS.

In the adult the ductus arteriosus of *Sus scrofa* not merely presents an occluded lumen, but the identity of the vessel as such is lost. However, one can often detect the site of the former lumen. The elastic tissue throughout the ductus instead of appearing in distinct fibers and lamellæ is broken up into fragments and may appear in small masses, the latter more especially at the site of the former lumen (figure 14). The whole ductus has undergone regression and is now essentially a fibro-elastic cord connecting up the pulmonary and systemic aortæ. Here and there unstriped muscle fibers remain. Small blood vessels supply the tissues of the retrogressed ductus.

SUMMARY.

A study of the histogenesis of elastic tissue in the embryonic ductus arteriosus of *Sus scrofa* is in accord with the theory that elastic fibrils are directly differentiated in the outlying portion of the protoplasm of the early connective tissue cell.

In the occlusion of the postfetal ductus arteriosus of *Sus scrofa* there is early a hypertrophy of the internal elastic membrane. Subsequently there takes place a marked delamination of the thickened internal elastic membrane in the production of new and independent elastic fibers and lamellæ. The formation of new elastic fibers from preformed elastic tissue is most abundant where the postfetal contraction of the ductus arteriosus is least marked. These new elastic fibers play an important part in the occlusion of the lumen of the postfetal ductus.

Aside from the extensive formation of elastic fibers from preformed elastic tissue, in the occlusion of the lumen of the postfetal ductus arteriosus of *Sus scrofa,* there are also some elastic fibrils formed from non-elastic elements, apparently from connective tissue cells.

In some recent preliminary work on ligations of the common carotid artery there was found, after an interval of from eight to twelve days, at some points between the ligatures, a slight but obvious cellular thickening of the so-called subendothelial stratum. Some of these connective tissue cells may have wandered from the

other coats of the vessel, through the inner elastic membrane into the subendothelial stratum; others may have proliferated from cells *in situ.* Specific stains revealed near the periphery of some of these cells, *i. e.,* in the outlying portion of the exoplasm, very delicate elastic fibrils, apparently the product of protoplasmic activity.

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EXPLANATION OF PLATES.

The first fourteen figures are photomicrographs of cross-sections of the ductus arteriosus. The sections illustrate the various stages in the occlusion of the lumen of the ductus and its obliteration as such. Figures 1 to 10 inclusive and 13 and 14 are from sections stained with either Weigert's elastic stain and counterstained with picrofuchsin, or some form of orcein stain. Figures 11 and 12 are from sections stained with hematoxylin and eosin. Figures 15, 16, and 17 are camera lucida drawings from Weigert-picrofuchsin stained sections.

PLATE 27.

FIGS. 1 to 3. Cross-sections of human ductus arteriosus from infants aged 30, 21, and 102 days, respectively. Note in figures 1 and 2 the pad-like thickening of the intima and the abundance of elastic tissue in this thickening. In figure 3 the inner elastic membrane is thickened at places, with denser laminæ bounding ectally and entally a finer network of elastic fibrils. The subendothelial stratum is also hyperplastic.

FIG. 4. Cross-section of a fetal ductus arteriosus from a pig 24 cm. long. Note the multilaminar condition of the inner elastic membrane on one side.

FIGS. 5 to 8. Cross-sections taken from various levels of the ductus arteriosus of a postfetal pig fourteen days old. In most of the sections the early, characteristic thickening and delamination of the inner elastic membrane is well shown. The multilaminar condition is well advanced in sections (figures 7 and 8). The latter are from the aortic thirds of the ductus. The pulmonary end of the ductus was even farther advanced in the production of new lamellæ from the inner elastic membrane (compare figure 9). In figure 5 a mass of blood is found in the lumen of the ductus, and from all appearances blood was flowing through the lumen of the vessel when the specimen was secured. The characteristic contraction so often seen in the pig ductus after birth is illustrated in figure 6.

Note especially the fine network of elastic fibers in the subendothelial stratum in the section represented in figure 8. The finer elastic fibrils are readily distinguished from the heavier lamellæ which are a product of the preformed inner elastic membrane.

PLATE 28.

FIG. 9. Cross-section from the pulmonary thirds of the ductus arteriosus from a pig fifteen days old. The lumen is more or less irregular, due to contraction, but a considerable amount of intimal hyperplasia would be necessary to occlude the lumen. The inner elastic membrane has already thickened and at places split into a number of new laminæ.

FIG. 10. Cross-section of the ductus arteriosus from a pig eight weeks old. The lumen of the ductus is entirely filled in with a mass of new tissue. Note the irregularity of the former lumen, and the new, heavy elastic laminæ formed from the old inner membrane. Here and there blood channels with definite walls and in some instances with well formed inner elastic membranes are found in the occluding mass. Note also the finer and less deeply stained network of elastic fibrils throughout the occluding mass.

FIG. 1.

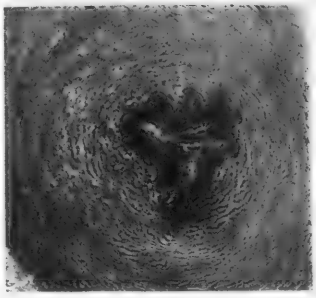

FIG. 5.

FIG. 2.

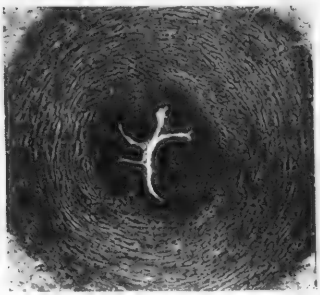

FIG. 6.

FIG. 3.

FIG. 7.

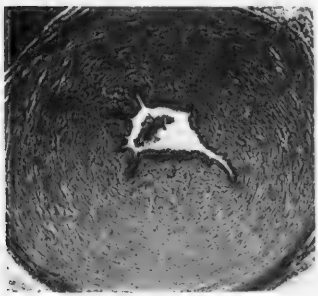

FIG. 4.

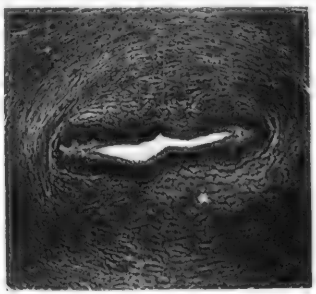

FIG. 8.

(Schaeffer: Behavior of Elastic Tissue in Postfetal Occlusion.)

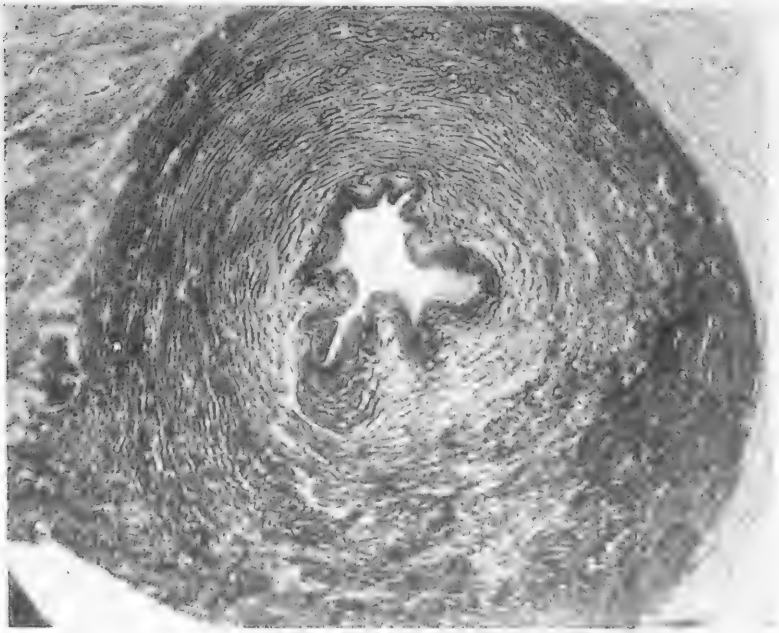

FIG. 9.

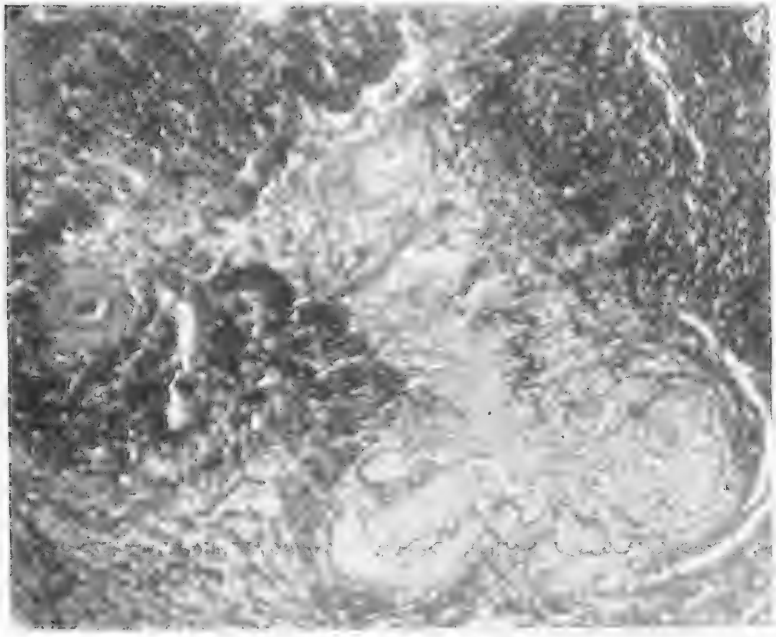

FIG. 10.

(Schaeffer: Behavior of Elastic Tissue in Postfetal Occlusion.)

FIG. 11.

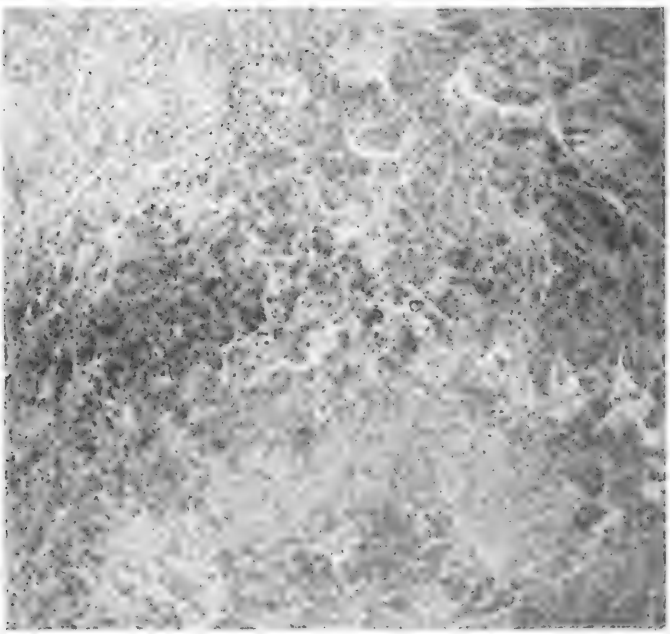

FIG. 12.

(Schaeffer: Behavior of Elastic Tissue in Postfetal Occlusion.)

FIG. 13.

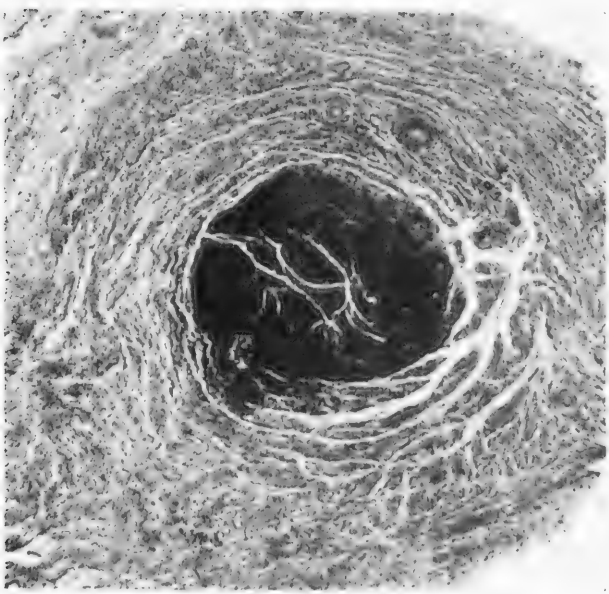

FIG. 14.

(Schaeffer: Behavior of Elastic Tissue in Postfetal Occlusion.)

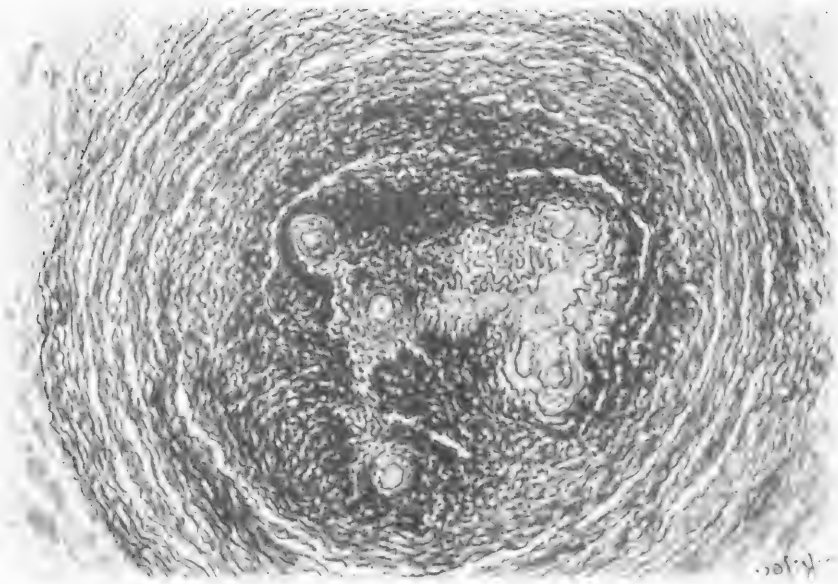

FIG. 15.

(Schaeffer: Behavior of Elastic Tissue in Postfetal Occlusion.)

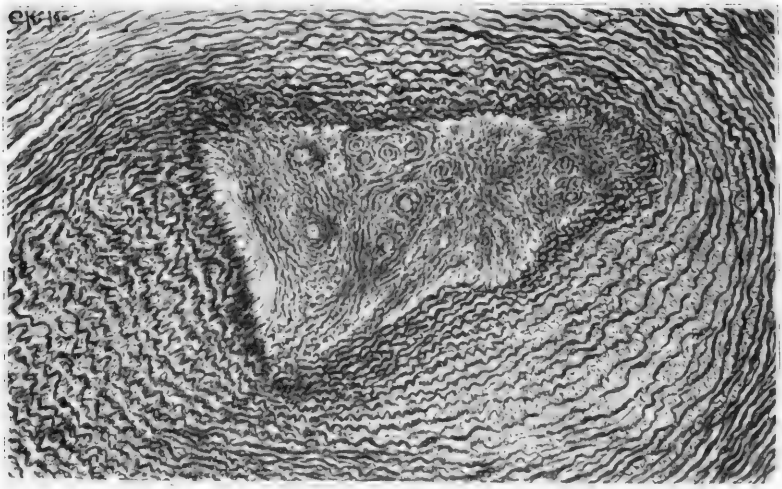

FIG. 16.

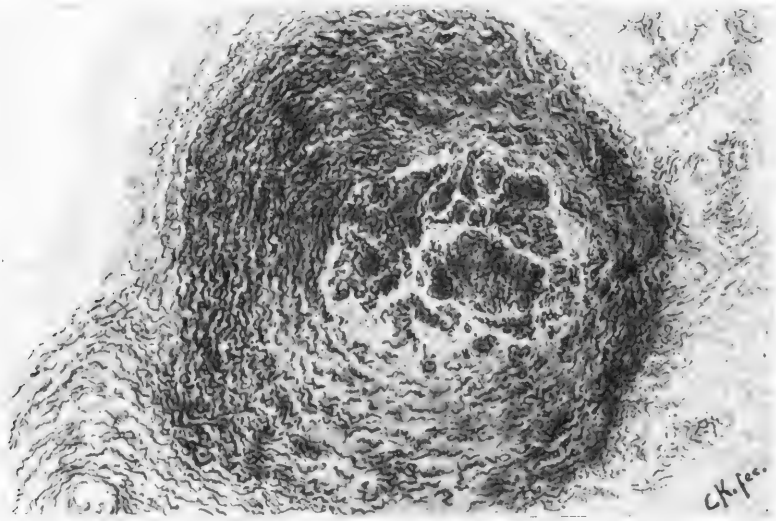

FIG. 17.

(Schaeffer: Behavior of Elastic Tissue in Postfetal Occlusion.)

PLATE 29.

FIG. 11. Cross-section of the ductus arteriosus from a pig eight weeks old. The section is stained with hematoxylin and eosin. Note especially the complete occlusion of the lumen of the vessel and the large number of blood channels in the occluding mass. The mass looks somewhat like an organized thrombus with subsequent canalization (compare figure 10). Both sections are from the same segment of the same ductus.

FIG. 12. Cross-section of the ductus arteriosus from a pig ten weeks old. The section is stained with hematoxylin and eosin. The lumen of the ductus is entirely occluded and obliterated. Note especially that in place of blood channels, illustrated in figures 10, 11, and 15, there are nests of cells, obviously the site of earlier vascular channels. Smaller vessels have now grown into the new tissue to supply it.

PLATE 30.

FIG. 13. Cross-section of the ductus arteriosus of a pig two days old. The section is stained for elastic tissue. Note the marked contraction of the lumen in this instance. The inner elastic membrane is already somewhat thickened and at places split into layers.

FIG. 14. Cross-section, stained for elastic tissue, of the ductus arteriosus from an adult pig. Note the great amount of broken up elastic tissue in the occluding mass in comparison with the amount remaining elsewhere in the retrogressed ductus.

PLATE 31.

FIG. 15. Camera lucida drawing of a Weigert-picrofuchsin stained section of the ductus arteriosus from a pig sixty days old. The delamination of the thickened inner elastic membrane is here well illustrated. Aside from the heavier elastic lamellæ in the occluding mass there are many finer elastic fibrils present, less deeply stained.

PLATE 32.

FIG. 16. Camera lucida drawing of a cross-section from the aortic thirds of the ductus arteriosus from a pig sixty-three days old. The section was stained with Weigert's elastic tissue stain and counterstained with picrofuchsin. Note the abundance and the disposition of the elastic tissue and the definite vascular channels in the occluding mass.

FIG. 17. Camera lucida drawing of a cross-section from the midportion of the ductus arteriosus from a pig eleven weeks old. Note the disposition of the elastic tissue at this time (compare figure 12).

INTESTINAL OBSTRUCTION.

III. The Defensive Mechanism of the Immunized Animal against Duodenal Loop Poison.*

By G. H. WHIPPLE, M.D., H. B. STONE, M.D., and B. M. BERNHEIM, M.D.

(*From the Hunterian Laboratory of Experimental Pathology, Johns Hopkins Medical School, Baltimore.*)

In previous communications (1, 2, 3) we pointed out the value of closed duodenal or intestinal loops in the study of some of the obscure features of intestinal obstruction. The closed loop may be likened to a volvulus in which there has been no vascular disturbance or obstruction, and the clinical picture in the two conditions may be identical. The closed loop usually employed in our work is produced in dogs by heavy ligatures placed just below the pancreatic duct and again just beyond the duodenojejunal junction combined with a posterior gastro-enterostomy. The intoxication which develops from the condition must be limited to two factors, bacterial growth and epithelial activity, as other factors such as gastric and pancreatic juice, bile, and products of food digestion are excluded.

Our published experiments show that dogs with closed duodenal loops will die in about two days from acute intoxication, and further that a substance can be isolated from the closed loops, which, if injected into a normal dog, will cause similar but more intense signs of intoxication. The picture is one of severe and fatal shock, —low blood pressure and temperature, vomiting and diarrhea with extreme splanchic congestion. Moreover, this toxic substance can be extracted from the mucosa of the closed duodenal loops and destruction of the mucosa prevents its appearance in the loops, indicating that the epithelium is essential to its production. The loop in question can be excised without any intoxication resulting therefrom.

* Received for publication, November 29, 1913.

Some of our early experiments led to the conclusion that a normal dog which had been injected intravenously with a sublethal dose of this toxic material became relatively immune, and could withstand much larger doses given by subsequent injection with little intoxication. This immunity can be demonstrated with little difficulty in spite of the varying reaction of normal dogs to the toxic material obtained from the duodenal loops. It was also noted that healthy dogs immunized by injection of the toxic substance can survive operation and the production of a closed duodenal loop for a period of six days or longer. The average duration of life in a non-immune dog after production of a closed duodenal loop is two days, the maximum four days. Further, the immune dogs do not show the early signs of intoxication which are so evident in the untreated dogs.

It seemed likely that this immunity might be explained by the presence of the protective ferments which appear in the blood after the injection of proteids and peptones (Abderhalden (4)). Repeated injection of sublethal doses of the poisonous material from duodenal loops showed that the blood after a suitable period was free from any ferments capable of dissolving various peptones as tested by means of the polariscopic method.[1] The material injected was obtained from closed loops, digested for many days at 38° C., heated to 70° C. for one half hour, centrifugalized, and filtered (Gooch crucible). It seems clear that the cleavage had been sufficient to destroy all peptones. Additional evidence is supplied by the observation that guinea pigs cannot be sensitized by the material so that the anaphylactic reaction may later be elicited. Guinea pigs sensitized by the blood of a closed loop dog will not react to the duodenal loop fluid prepared as outlined above.

Evidence is submitted below to show that the poison found in the closed loop of the dog or cat, and in the human intestine in acute obstruction, may be the same. The poisons have the same toxic effects and may all be used to immunize dogs against subsequent lethal doses of the material from a closed duodenal loop. Further it is noted that a dog (No. O-66) which recovered from a simple obstruction was extremely resistant to the intoxication of a closed

[1] These observations were made by Dr. E. K. Marshall.

duodenal loop, exactly like a dog which had been immunized with loop poison. This fact surely indicates that a poison is absorbed under these conditions, and also that the poison present in simple obstruction and in the closed loop may be identical.

The poison found in the closed loops is a resistant body (1). It is evident that the immune dog develops new power or increases its normal capacity to destroy this poison. The experiments below show that the immune serum is inert but that the protective agent resides in the organ cells and extracts. The liver, spleen, and lungs are very active in destroying the duodenal loop poison when a mixture is incubated for a few hours or days. The clear filtrate from the digested immune liver can destroy the poison after autolysis for hours, indicating possibly some ferment as the active agent.

Normal tissues act on the poison very slowly, and weeks may be required to show any definite destruction of the toxin. Normal liver acts more quickly than other normal organs, but is far weaker than the immune liver. This fact may be taken as evidence of the method of defense of the body against this poison. This protective body or ferment is not a stable factor, but may disappear; so too the acquired immunity of dogs may vanish after a period of weeks or months.

METHOD.

The method will be given here in outline; the full account will be found in previous publications. The material obtained from the duodenal loop is allowed to undergo autolysis with chloroform and toluol for several days at 38° C., after which it is heated at 60° to 70° C. for one half hour or longer, centrifugalized at high speed for several minutes, and the supernatant fluid filtered through filter paper or asbestos (Gooch crucible). The filtrate may be faintly acid or alkaline. Unless otherwise stated the material obtained from the intestinal loops or mucosa is treated in this manner before injection. The operative technique has been described and illustrated in a former publication. It was found that intraperitoneal injection of this material caused some inflammation and that subsequent operation was not infrequently followed by peritonitis, resulting from the slight soiling of the peritoneum during the production of the gastro-enterostomy. Intravenous injection is much more satisfactory when it is desired to produce an immunity.

EXPERIMENTAL OBSERVATIONS.

DOG IMMUNIZED TO DUODENAL LOOP FLUID. LETHAL DOSE. SURVIVED.

Dog O-31.—Mongrel hound, male.

Jan. 29, 1913. 12 M. Intravenous injection of 20 c.c. of duodenal fluid (dog

O-12). 1 P. M. Muscular tremors and intoxication but no vomiting. 3 P. M. Repeated attacks of vomiting. Dog looks rather sick.

Jan. 30. Dog appears normal.

Feb. 1. 2 P. M. Intravenous injection of 15 c.c. of duodenal fluid (dog S-51-54). This duodenal fluid, 20 c.c. in amount, was fatal to a 15 pound dog in four and one half hours. 4 P. M. Repeated vomiting and much fluid diarrhea.

Feb. 2. Dog has recovered completely and has a good appetite.

Feb. 4. Dog in good condition; weight 22¼ pounds. 3 P. M. Intravenous injection of 20 c.c. of duodenal fluid (dog S-51-54). 3.30 P. M. Vomiting and much fluid feces. 4.30 P. M. Dog improving. Pulse is much stronger; no vomiting.

Feb. 6. Dog is in good condition; weight 21¼ pounds. 10.30 A. M. Temperature 38.2° C. Ether anesthesia and kymograph observation. Intravenous injection of duodenal fluid, 30 c.c. (dog O-26). This fluid, 30 c.c., was fatal in seven hours to a dog weighing 25 pounds. The kymograph record (dog O-31) shows initial drop in blood pressure as usual, followed by a slow secondary drop always seen in fatal poisoning. At the end of the kymograph record the temperature was 37.3° C. Following recovery from ether, vomiting and diarrhea were marked. 12.30 P. M. Temperature 39.6° C. 2.30 P. M. Dog has vomited and had some dark fluid feces; appears much intoxicated but can walk about. 4 P. M. Temperature 39.7° C.

Feb. 7, 10 A. M. Dog is rather sick, but this is due to an extensive phlegmon developing about the neck wound involving the region of the shoulder. Temperature 39° C. 12 M. Bled from carotid. Ether anesthesia.

Autopsy.—Performed at once. There is an extensive area of inflammation at the root of the neck. Thorax and lungs negative. Liver rather pale. Gastro-intestinal tract normal. Blood serum from this dog used below to show the absence of ferments capable of destroying duodenal poison. This serum together with a normal control showed no ferment activity after digestion with peptone and gelatin, with the polariscopic method (Abderhalden).

INCREASED RESISTANCE TO DUODENAL LOOP FLUID.

Dog D-38.—Small pup; weight 10 pounds.

Jan. 16. Intravenous injection of duodenal loop fluid from a cat. This caused considerable intoxication, vomiting but no diarrhea.

Jan. 23. Dog is noisy and active.

Jan. 24, 10 A. M. Duodenal fluid, 20 c.c. (dog O-11), given intraperitoneally. 12 M. Frothy vomitus below cage, but no diarrhea.

Jan. 25. Dog is better, but abdomen is tender.

Jan. 28. Dog in good condition; weight 10 pounds. 10.30 A. M. The duodenal fluid was standardized. 20 c.c. killed a dog weighing 10½ pounds in four hours, with typical signs of acute intoxication, the experiment being done at the same time with this experiment. A kymograph record (dog D-38) was taken during this intravenous injection of 32 c.c., which is a 50 per cent. increase above the known lethal dose. The fluid caused a profound drop in blood pressure followed by a slow rise and slowing of pulse.

12.30 P. M. Dog badly shocked. Passes a little fluid feces. Pulse just palpable. Respiration slow; appears fatally poisoned. 3 P. M. Dog is badly shocked

but has improved slightly, and the diarrhea has ceased. 4 P. M. Passes a large amount of fluid feces rich in mucus and bile. Bile-stained vomitus abundant. Pulse is much stronger. 6 P. M. Condition about the same.

Jan. 29, 9 A. M. Found dead.

Autopsy.—Beginning peritonitis over a large intussusception involving the middle portion of the small bowel. Thorax negative. Viscera are not much congested. Jejunum contains a good deal of fluid. The mucosa is perhaps slightly pinker than normally. It is probable that this dog would have survived this large dose of toxic material but for the intestinal intussusception, brought on undoubtedly during the active peristalsis which always follows the injection of this poisonous material.

The preceding experiments (dogs O–31 and D–38) show that a considerable grade of immunity may be established against the loop poison even by a single sublethal dose. Other experiments given below bring out the same point (dog O–51). The following experiment (dog O–43) shows again the increasing immunity following sublethal doses of the poison; and it appears that it is transient and may drop to a low level or disappear completely after a period of many weeks.

IMMUNITY TO DUODENAL LOOP FLUID DISAPPEARS IN THREE MONTHS.

Dog O-43.—Strong fox-terrier, male; weight 25½ pounds.

Feb. 17, 11 A. M. Duodenal fluid, 10 c.c., given intravenously (dog O-33-34). 12 M. Dog looks shocked. 2 P. M. No diarrhea; no vomitus. Dog improving.

Mar. 19, 11 A. M. Duodenal fluid (X), 14 c.c., given intravenously. 10 c.c. of this fluid had poisoned fatally within four hours a normal dog weighing 25 pounds. 4 P. M. Much vomiting and profuse diarrhea. The fluid stools are rich in mucus, as seen in fatal poisoning. 5 P. M. Pulse is weak, but dog is evidently recovering.

Mar. 20. Dog has recovered completely.

Mar. 24, 12 M. Duodenal fluid, 20 c.c., given intravenously (dogs O-33 and O-34). 14 c.c. of the fluid poisoned a dog fatally in six hours (dog O-38; weight 21 pounds). 3 P. M. Dog has vomited repeatedly. Much fluid diarrhea, but does not look badly shocked. 5 P. M. Diarrhea continues with tenesmus and bloody mucus. Animal looks sick.

Mar. 29. Dog in normal condition.

Apr. 12. Dog in fairly good condition; weight 22 pounds. 12 M. Duodenal fluid (X), 14 c.c., given intravenously. 2 P. M. Dog has had a little diarrhea; no vomiting. Pulse fair. 8 P. M. Dog eats and looks well. Pulse fair. 8 P. M. Dog eats and looks well. Pulse normal. The poisoning is obviously much less marked at this time than in the previous month.

July 25. Dog has mange, but is in fair condition; weight 22.5 pounds. 11 A. M. Dog given a lethal dose of duodenal fluid, 150 c.c. (dog O-52). This fluid had been boiled and filtered and tested on a normal dog (No. O-101), weighing

24 pounds. The same amount of fluid used, 150 c.c., caused in the control dog fatal intoxication in eight hours with typical findings of an acute intoxication. 12.30 P. M. Dog has had one fluid stool and shows a slow deep respiration. 2.30 P. M. Dog is severely shocked and more soft feces are passed. 5 P. M. Diarrhea continued. Dog is very sick and died shortly after this observation.

Autopsy.—July 26, 9 A. M. Heart contained blood clots. Lungs show a great deal of edema. Liver, spleen, and stomach normal. Duodenum shows a mottled pink and red mucosa. Ileum mucosa is pale and the small bowel contains a great deal of fluid feces. Kidneys show a moderate grade of chronic nephritis. The resistance of this dog to duodenal poisoning at this time was not above normal and very much less than during the period three months earlier, when lethal doses of poison were survived with relatively little intoxication.

IMMUNITY WITH DOG AND HUMAN MATERIAL.

Dog O-90.—Black and tan male; weight 10¾ pounds.

May 24. Duodenal fluid, 50 c.c., (dog O-77) given intravenously. This caused some vomiting and diarrhea, but the dog made a good recovery.

June 24. Weight 11 pounds. 3 P. M. Human obstruction fluid (3921), 50 c.c., given intravenously. This did not cause severe intoxication, and it was slightly toxic to normal dogs.

June 25, 9 A. M. Dog is active and lively.

June 26, 12 M. Dog is in good condition; weight 10¼ pounds. Human obstruction fluid (jejunum 3759), 30 c.c., given intravenously. This fluid was very toxic, 30 c.c. being fatal to dog O-82 in five hours. Weight 13¼ pounds. 4 P. M. A few soft stools but no diarrhea. No vomiting. No evidence of shock.

June 27. Dog is active and hungry.

The preceding experiment (dog O–90) and one cited below (dog S–90), as well as other observations, show that the poisonous material present in the closed duodenal loops is similar to that present in human intestinal obstruction. Examples need not be multiplied but it can be stated that animals can be immunized against the duodenal loop poison of their own species (*e. g.* dog) by the material obtained from the closed loops of other species (*e. g.* cat), or from intestinal obstruction in man.

INTESTINAL OBSTRUCTION RELIEVED GIVES IMMUNITY TO CLOSED DUODENAL LOOP FLUID.

Dog O-66.—Yellow mongrel, male; weight 14 pounds.

Mar. 28. Ether anesthesia and operation; simple intestinal obstruction. Small intestine closed by ligature midway between the pylorus and ileocecal valve.

Mar. 31. Dog is dull and sick.

Apr. 1. Intravenous injection of a mixture containing ascitic fluid and organ extracts from immune dog.

Apr. 3. Dog seems slightly better.

Apr. 4. Dog has passed feces and is obviously improving. The ligature has probably cut through and established the continuity of the intestinal tract.

Apr. 5. Dog has had diarrhea and appears in good condition.

Apr. 10. Dog appears normal; weight 13¾ pounds.

Apr. 11. Exploratory operation under ether anesthesia. The ligature has cut through, and the lumen of the small gut was practically normal at this point, but the intestine above the site of ligation is definitely dilated and its walls are hypertrophied. A closed duodenal loop was done in the usual manner, together with a posterior gastro-enterostomy.

Apr. 12. Dog shows no evidence of intoxication.

Apr. 14. Weight 11¼ pounds. No signs of acute intoxication.

Apr. 15. Dog has distemper with purulent discharge from nose. Pulse is good and strong. Weight 11¼ pounds. 4 P. M. Given intravenously ascitic fluid, 500 c.c., from dog 12-44 (experimental passive congestion).

Apr. 16. Dog appears fairly well and walks about. Temperature 39.1° C. Weight 10½ pounds.

Apr. 17. Dog eats a little food and is stronger. No evidence of intoxication. Weight 10½ pounds.

Apr. 18. Dog is in fair condition; weight 10¾ pounds.

Apr. 19. Dog is improving and passed a solid stool. Weight 12 pounds.

Apr. 21. Condition remains the same. There is evident distemper. Temperature 39.1° C. Weight 12 pounds.

Apr. 22, 11 A. M. Weight 12¼ pounds. Ether anesthesia, exploratory laparotomy. The upper ligature had cut through, and the lower ligature was intact. The lumen here was completely obstructed. Gastro-enterostomy perfect. The loop was decidedly thickened and dilated, but contained only 30 to 50 c.c. (estimated). The duodenal loop was again isolated by means of cutting across the bowel and inverting the ends, as has been described in previous papers. Such dogs died as a rule within twenty-four hours and rarely survived thirty-six hours.

Apr. 23, 3 P. M. Dog curled up quietly. Weight 11½ pounds. Temperature 39.3° C.

Apr. 24, 12 M. Dog looks sick. Pulse is weak. Temperature 38.1° C. Weight 11½ pounds. 5 P. M. Died.

Autopsy.—Performed at once. The peritoneal cavity contains 200 c.c. of purulent, foul smelling fluid. There was an abscess at the lower end of the duodenal loop which had ruptured, causing general peritonitis. Thorax, heart, lungs, spleen, and liver negative. Stomach collapsed. Small intestine collapsed and contracted tightly around the root of the mesentery. The loop is small and contains thick, tenacious paste. Its mucosa, except for that covering the inverted ends, is normal.

The preceding experiment (dog O–66) is worthy of careful study and analysis. A simple obstruction of the small intestine presented the usual picture of intoxication, which was somewhat relieved by an injection of a solution of organ extracts from an immune dog. We shall not discuss this point here, and merely state that by about the fifth or sixth day there was improvement. It

was obvious that the obstruction had been relieved by the cutting through of the ligatures.

An exploratory operation two weeks after the first operation showed that the obstruction had been completely relieved, but the jejunum was dilated and hypertrophied. It may be supposed that this dog had been immunized by absorption from his own mucosa during the simple obstruction.

At this time a closed duodenal loop was made as usual and it is noteworthy that the dog appeared in no way intoxicated during the next few days, although he received no fluid infusions and no treatment of any kind, except on the fourth day. The closed loop was present for eleven days, and at this time a second exploratory operation showed an enlarged and thickened duodenal loop containing fluid. The lower ligature was intact and the upper had cut through allowing the escape of fluid on the application of pressure. In rare instances in very vigorous dogs we have observed the cutting out of a ligature with leakage of the loop fluid into the bowel above or below. These animals die, but may survive four or five days. The loop in this dog was securely closed the first few days and later became partly drained. It should be recalled that the majority of untreated dogs with duodenal loops draining externally die in three to six days.

There is no escape from the conclusion that this dog had a strong immunity toward the intoxication resulting from a closed duodenal loop, and the immunity developed as a result of a preceding simple intestinal obstruction, which emphasizes the fact that the intoxication of simple obstruction is similar or probably identical with that developing in association with a closed duodenal loop.

IMMUNIZED DOG WITH CLOSED DUODENAL LOOP. KILLED AFTER SIX DAYS.

Dog S-51.—Strong adult male; weight 15 pounds.

May 14. Dog was given intraperitoneally 30 c.c. of material obtained from the closed duodenal loop mucosa of dog S-29. This caused very little intoxication.

May 15. Dog seems normal.

May 17. 11 A. M. Dog was given 28 c.c. of material obtained from a closed duodenal loop (dog S-38). This likewise gave little indication of intoxication.

May 18–21. Dog is normal and eats well.

May 22, 12 M. Dog was given intraperitoneally duodenal loop fluid, 30 c.c. of clear filtrate, prepared as usual, obtained from dog S-11. 2 P. M. Dog had some diarrhea but no vomiting.

May 29, 3 P. M. Ether anesthesia and operation as usual with isolation of closed duodenal loop.

May 30, 1 P. M. Temperature 38° C. Dog eats; no vomiting and no diarrhea.

May 31, 10 A. M. Temperature 38.8° C. Dog eats well and seems in excellent condition. 5 P. M. Temperature 39° C. Dog refused food.

June 1, 10 A. M. No vomiting and no diarrhea, but refused food. Temperature 39° C. Given milk by stomach tube. 5 P. M. Temperature 38.6° C. Condition about the same.

June 2, 12 M. Dog has considerable diarrhea and drinks water eagerly; pulse good. Temperature 37° C. Given milk again by stomach tube, which was followed by vomiting. Dog has lost considerable weight and strength.

June 3, 10 A. M. Dog lies quietly in the cage. Temperature 36° C. Dog has had some diarrhea, the fluid being streaked with a little blood. Pulse is slow but of good volume and tension. 2 P. M. Temperature 35.5° C. Given 200 c.c. of salt solution subcutaneously, as animal will not retain fluids given by the stomach.

June 4, 10 A. M. Dog appears improved. Temperature 37.5° C. The pulse is strong and full. No diarrhea, and stools are pasty. 12 M. Given milk by stomach tube. 2.30 P. M. Pulse strong. Temperature 37° C. Given ether and bled from carotid. The blood pressure was good at this time. Blood showed a good deal of hydremia, giving about four fifths by volume of serum after centrifugalization. The dry weight of the blood at this time was 12.4 and 12.3 per cent., in parallel determinations.

Autopsy.—Performed at once. Heart, lungs, thorax, and peritoneal cavity normal. Spleen is pale and fibrous. Pancreas and kidneys are normal. The stomach contains bile-stained fluid and milky curds. The mucosa is normal. Small intestine shows a pale normal mucosa, in which hookworms are rather numerous. The duodenal loop is large and flabby. Its ends are closed tightly and it contains about 150 c.c. of canary yellow, purulent material. The mucosa is slightly swollen and pinkish, but intact throughout. Three hookworms are alive and active and adherent to the mucosa.

Microscopical Examination.—Spleen is atrophic. Kidneys, liver, duodenal loop, jejunum, and stomach mucosa are all normal.

IMMUNIZED DOG WITH CLOSED DUODENAL LOOP. KILLED AFTER FOUR DAYS.

Dog S-90.—Old mongrel, female; weight 12½ pounds.

July 16, 3 P. M. Ether anesthesia and intravenous injection of fluid obtained from a case of human intestinal obstruction. This material had undergone autolysis in the usual way for several days, was heated at 60° to 70° C. for two hours, centrifuged, and filtered. 20 c.c. of this broth-like filtrate were given intravenously and the ether removed at once. The duration of the anesthesia was only about ten minutes. 5 P. M. Pulse is just palpable and dog appears much shocked. Dog has vomited twice and passed one semifluid stool. 8.30 P. M. Pulse is good and dog is much improved. No diarrhea.

July 17, 9 A. M. Dog will not eat.

July 18, 10 A. M. Dog appears rather sick; in the afternoon ate some food and seems better.

July 21. Dog seems well.

July 22, 3 P. M. Ether anesthesia and operation as usual with isolation of a closed unwashed duodenal loop.

July 23, 9 A. M. Temperature 39° C. Dog is active. 5 P. M. Temperature 38.9° C.

July 24, 2 P. M. Rectal temperature 38.4° C. Dog drinks water but will not eat.

July 25, 1 P. M. Rectal temperature 38.4° C. Dog has lost weight and strength and refuses food.

July 26, 10 A. M. Rectal temperature 38.7° C. Pulse is good. 3 P. M. Rectal temperature 38.6° C. Dog is fairly strong, but has lost a good deal of weight. Given ether and bled from carotid. The pulse pressure is strong, and there is no indication of a severe intoxication. It is probable that this animal would have lived one or two days longer.

Autopsy.—Performed at once. Peritoneal cavity, thorax, heart, and lungs are all normal. Spleen and kidneys are normal. Liver is pale brownish in color, but otherwise normal. The duodenal loop is not distended. The ends are tightly closed. It contains about 10 c.c. of pasty, creamy material, which sets into a sort of jelly on contact with water. This is squeezed out and the mucosa scraped off as usual. The mucosa is normal and intact throughout. There are no ulcers and no submucous hemorrhages. Stomach and duodenum above the ligature contain bile-stained fluid. The mucosa is pale and intact. The small intestine contains bile-stained fluid, and its mucosa is pale and intact. The gut is uniformly constricted throughout.

The two preceding experiments (dogs S–51 and S–90) show a type of immunity which develops following the injection of the toxic material in sublethal doses. The results are the same whether material from closed loops in dogs or from intestinal obstruction in man is used to immunize the animals. The dogs with the closed loops do not show the usual acute signs of intoxication seen in dogs not previously injected. They survive often far beyond the maximum period for untreated dogs with closed loops. Hence the poison found in the closed loops is the cause of the intoxication resulting from their experimental production. A similar poison is found in human intestinal obstruction and presumably is concerned with the intoxication found in this condition.

The two next experiments (dogs S–72 and S–70) illustrate some of the difficulties in the work with the closed-loop dogs. The immunity following repeated doses of the poison will protect effectively against the loop intoxication, but not against peritonitis. The closed loops may behave very differently under various conditions. At times in a fatal case the loop will be found collapsed and containing only a few cubic centimeters of thick, pasty material. These cases are favorable for immunity work. If an immunity is

established in such a dog the animal may live more than double the usual period, but in most animals there is a rapid accumulation of fluid in the loop which produces tension and favors injury and ulceration of the mucosa which may effect a perforation. Perforation, of course, causes peritonitis with rapid death, and adds a confusing factor to the picture.

IMMUNIZED DOGS WITH CLOSED DUODENAL LOOP. DEATH IN FOUR DAYS WITH PERITONITIS.

Dog S-72.—Strong adult fox-terrier, male; weight 22 pounds.

June 21, 11 A. M. Dog was given 22 c.c. of duodenal loop fluid obtained and prepared as usual (dog S-65). This was injected intraperitoneally and caused no pain. 1 P. M. Dog has vomited once, but there is no diarrhea.

June 22. Dog is perfectly normal and eats well.

June 24, 5 P. M. Dog given intraperitoneally 25 c.c. of duodenal fluid (dog S-51).

June 25, 9 A. M. Dog is normal.

June 27, 3 P. M. Ether anesthesia and operation with isolation of closed duodenal loop in the usual way with washing out of contents of loop.

June 28. Dog is quiet and refuses food.

June 29, 10 A. M. Dog is in good condition. Pulse strong. Rectal temperature 38.5° C. Muscular tremors are noted. 4 P. M. Temperature 39° C. Given one half pint of milk. 5 P. M. Dog has vomited milk and bile-stained material. Small amount of soft blood-tinged feces are passed. Dog seems obviously intoxicated.

June 30, 12 M. Dog again given milk with one egg by stomach tube. Weight 19 pounds. Rectal temperature 40° C. Pulse strong and regular. 1 P. M. Dog vomited bile-stained coagulum.

July 1, 9 A. M. Condition about the same. Temperature 39° C. 11 A. M. Dog is given milk which is vomited at once, and later a little water which is also vomited immediately. 2 P. M. Pulse is slow and weak. Temperature 38.8° C.

2.15 P. M. Death. Blood obtained a few minutes after death showed dried weight of 22.3 and 22.4 per cent., in parallel determinations.

Autopsy.—Performed at once. Thorax, heart, lungs, and spleen are all normal. The peritoneal cavity contains some purulent, turbid fluid, about 20 c.c. in amount. The serous surfaces are injected and covered with tiny ecchymoses. It is found that there had been leakage from the lower puncture wound and lower ligature which had cut into the gut wall. Jejunum shows a mottled, pinkish mucosa with little injection. The ileum is quite normal and contains soft yellow feces, and fecal material slightly stained with blood. Liver is considerably injected. Kidneys and stomach are normal. The loop contains about 20 c.c. of slate-colored, thick material having a fecal odor. The mucosa is intact throughout and normal except for a little injection.

Dog S-70.—Small pup, male; weight 11 pounds.

June 20, 11.30 A. M. Ether anesthesia with kymograph observation. The material was obtained from the intestinal mucosa (dog S-66), a case of simple

intestinal obstruction. The intestinal mucosa, washed and scraped off, was allowed to undergo autolysis for six days, heated at 65° C. for one hour, centrifugalized, and filtered. 55 c.c. of this clear filtrate caused the usual drop of the blood pressure with return to normal. 12.20 P. M. Dog removed from kymograph. 1 P. M. Dog passed a semifluid stool. 2.30 P. M. Diarrhea is marked and dog severely shocked. Pulse is fair. 5 P. M. Dog is weak. Diarrhea persists.

June 21, 9 A. M. Dog seems normal and eats vigorously.

June 23. Condition is normal.

June 24, 12 M. Dog is normal; weight 11½ pounds. 12.30 P. M. Ether anesthesia and kymograph observation. The material was obtained from dog S-46 (drained duodenal loop). 90 c.c. of this material was given intravenously, causing an initial drop of blood pressure followed by a rise above normal. 1 P. M. Dog removed from kymograph. 3 P. M. Dog is much shocked: vomiting and diarrhea are marked. 5 P. M. Dog is quite sick. Muscle tremors are marked. Pulse is fair, and diarrhea continued.

June 25, 9 A. M. Dog seems well.

July 11, 3 P. M. Dog in excellent condition. Ether anesthesia and operation as usual, with isolation of closed unwashed duodenal loop.

July 12, 9 A. M. Dog seems well. 4 P. M. Condition the same.

July 13, 9 A. M. Dog has vomited. Rectal temperature 38.5° C. Pulse is strong. 4 P. M. Dog has passed solid and semifluid feces. 9 P. M. Pulse regular and strong.

July 14, 10 A. M. Dog seems pretty well. Temperature 38.3° C. Pulse regular, but tension is poor. Vomitus is present under the cage, and partly digested food is present in it. 5 P. M. Dog is very weak and toxic. Pulse is of low tension. Given 200 c.c. of water and vomited immediately. Temperature 38.2° C.

July 15, 9 A. M. Pup is very toxic. Pulse is weak. Rectal temperature 39.4° C. Vomited at once water given by stomach tube. Muscle tremors are present. 9.30 A. M. Death.

Autopsy.—Performed at once. Thorax and heart are normal. Lungs show a few small patches of consolidation along the edges of the left lower lobe. Spleen is negative. Liver is congested. The peritoneal cavity contains a little thin, purulent material, about 5 c.c. in amount. The surfaces are injected and show scattered ecchymoses. The leak from which the peritonitis developed is about the upper ligature where a tiny perforation and pocket of pus is found. The loop is not distended and contains only 20 c.c. of rather thick fluid. The mucosa is quite intact, slightly injected, and shows no ulcers and no hemorrhage. The stomach contains bile-stained fluid. The jejunum shows a pinkish red mucosa, with definite injection of the villi. Considerable fluid is present. The ileum shows a very faint pinkish mucosa. The large intestine and kidneys are normal.

IMMUNE SERUM INACTIVE TO DUODENAL LOOP POISON.

Dog O-31.—Mongrel hound; weight 21 pounds. This dog (see history above) was given repeated injections of duodenal loop fluid and survived a known lethal dose. The blood after defibrination was centrifugalized and 65 c.c. of blood serum were obtained. The red blood cells were then washed with salt solution, centrifugalized, and the washings added to the serum, making 130 c.c. in all.

To this was added duodenal loop fluid (dog O-26), 30 c.c., an amount which was known to be a lethal dose as tested on other animals. This mixture of immune dog serum and duodenal fluid together with chloroform and toluol was incubated for twenty hours at 38° C., and after heating and filtration was injected intravenously into dog O-37, a mongrel Irish terrier, male, weighing 13½ pounds.

Feb. 2, 9 A. M. Ether anesthesia and kymograph record during injection. There was no immediate depression of blood pressure and obviously the incubation with serum neutralizes or destroys this substance, causing the acute primary drop in blood pressure. There was a remarkable secondary fall in blood pressure coming on after about twenty minutes accompanied by great slowing of pulse. The dog passed fluid feces at the end of fifteen minutes and vomited before coming out of the anesthesia. 1.30 P. M. Death.

Autopsy.—The findings are in every respect typical of acute poisoning by the duodenal loop fluid.

IMMUNE SERUM DOES NOT DESTROY DUODENAL LOOP POISON.

Dog 12-34.—Small pup; weight 9 pounds.

Jan. 9. Intravenous injection of material from closed intestinal loop of cat. This caused a moderate grade of intoxication with diarrhea.

Jan. 14. Complete recovery. Ether anesthesia and bleeding from carotid. The blood serum gave negative results for the ferments described by Abderhalden, with peptone and the polariscope method. The remaining blood was then defibrinated and 70 c.c. of serum were obtained.

Autopsy.—Normal organs throughout. Blood, 70 c.c., plus duodenal fluid (dog O-11), 50 c.c., were placed in the incubator with toluol and chloroform for twenty-four hours at 39° C. There was slight turbidity at the end of this time. The fluid was heated to 55° C. for one half hour, centrifugalized, and filtered. This mixture, 115 c.c., was given intravenously to dog O-23, a strong adult fox terrier, weighing 16 pounds. 10.30 A. M. Ether anesthesia and kymograph record during the entire period of injection. There was no sudden drop in blood pressure and no marked drop during the period of observation (one half hour). A well marked erythema of the skin appeared over the legs and abdomen. 2 P. M. Dog is prostrated and has vomited. 3.30 P. M. Death.

Autopsy.—The findings were typical of acute poisoning by duodenal loop fluid, described and illustrated in previous publications (2).

Dog O-22.—Small pup; weight 7 pounds.

Jan. 11, 12 M. Human mucosa (3782). Autolysis for twelve weeks. Heated at 95° C. for thirty minutes; no precipitate; filtered. 95 c.c. intravenously gave little evidence of severe intoxication. 2.30 P. M. Dog appears fairly well. No diarrhea.

Jan. 12. Dog is normal.

Jan. 20. Bled for ferment tests. The Abderhalden tests with this blood serum were negative, as above, for the presence of protective ferments. Blood serum, 50 c.c., was combined with blood serum of dog 12-30 to be used later.

Dog 12-30.—Fox-terrier, male; weight 14½ pounds.

Jan. 11. Human intestinal contents treated as above (dog O-22). 40 c.c. given intravenously caused little reaction.

Jan. 12. Dog is normal.

Jan. 20. Ether anesthesia and bleeding. This blood serum was tested with negative result for the Abderhalden ferments. The blood serum (110 c.c.) combined with that of dog O-22 (50 c.c.) was added to duodenal loop fluid of dog O-11 (50 c.c.). This mixture was incubated together with chloroform and toluol for 22 hours at 39° C., then heated for one half hour at 60° C., and filtered. This clear brown fluid, 205 c.c., was given intravenously to dog O-27, a fox terrier, weighing 12 pounds.

Dog O-27.—Jan. 21, 12 M. Ether anesthesia. Kymograph observation showed a slow transient drop during injection, with a tendency to return to normal within thirty minutes. 3 P. M. Pulse is good. No evidence of shock. Has passed one solid stool. 8 P. M. Found dead, but quite warm. Fluid feces and vomitus abundant under cage.

Autopsy.—Performed at once. Heart still fibrillating. Blood clots slowly. Lungs show patches of hemorrhagic edema. Spleen is greatly enlarged. Liver is huge and deep purple in color, and very tense.

Mesenteric vessels are greatly dilated. Stomach contains a little fluid and shows a pale mucosa. Duodenum shows engorgement of the villi, but is contracted. The small intestine contains much blood-tinged fluid. The mucosa is not deep purple, but is mottled pink and red.

The three preceding experiments (dogs O–31, 12–34, and 12–30) show that the serum of dogs immunized by sublethal doses of the poison will not neutralize or destroy the duodenal loop poison. This contrasts strikingly with the observations given below dealing with immune organs and organ extracts which can destroy this poison when the mixture is incubated for a short space of time. The protective ferments described by Abderhalden play no part in this reaction and were absent in all cases examined.

LIVER, SPLEEN, AND LUNGS OF IMMUNE DOG DESTROY DUODENAL LOOP POISON.

Dog O-51.—Mongrel female; weight 15½ pounds.

Mar. 15. Duodenal loop fluid digested with mucosa of immune dog. The filtrate was given intravenously with little evidence of intoxication.

Mar. 16. Dog is normal.

Mar. 19. Duodenal fluid (X), 20 c.c., given intravenously. This caused a little vomiting and a slight amount of diarrhea, but no grave intoxication. The dog has evidently a considerable grade of resistance as this amount of fluid contains more than enough toxin to kill a normal dog weighing 25 pounds.

Mar. 20. Dog rather weak, but is recovering.

Mar. 22. Dog is normal, and eats as usual. Weight 13 pounds. Ether anesthesia, bleeding, and perfusion with 0.9 per cent. salt solution to wash out all the blood from the organs which are normal in all respects. The following extracts and emulsions were made with the addition of a standardized duodenal loop fluid, of which 10 c.c. was fatal to a normal dog weighing 25 pounds in a period of four hours.

(1) Duodenal loop fluid (X), 20 c.c., plus washed mucosa from small intestine, 50 c.c. Dilution with water, toluol, and chloroform; autolysis for five days; heated at 60° C. for one hour. This fluid mixture caused fatal and typical intoxication in a period of ten hours (dog O-64 below). It is probable that some of the poison was destroyed.

(2) Duodenal fluid (X), 20 c.c., plus whole spleen ground up with sand and made up to a thin emulsion with water, toluol, and chloroform. Autolysis for three days. Heated at 60° C. for two hours followed by centrifugalization. This fluid mixture caused no acute intoxication (dog O-63 below) and the poison had been destroyed.

(3) Duodenal loop fluid (X) plus liver, 250 gm., ground up with sand, extracted in Buchner press, and about 80 c.c. of the thick juice obtained. This was diluted to 190 c.c., centrifugalized, and filtered, giving 160 c.c. of buff colored filtrate. 50 c.c. of this liver juice plus 15 c.c. of duodenal fluid (X) given at once intravenously into a normal dog. The mixture had stood only a few moments before the injection. This caused a fatal and typical intoxication after four hours (dog O-56).

(4) Duodenal fluid (X), 20 c.c., plus liver juice, 50 c.c., prepared as in (3), plus toluol and chloroform. Autolysis for forty-four hours, heated at 60° C. for one half hour, and centrifugalized, giving a clear odorless broth. The entire amount was given intravenously to dog O-57 (see below) and caused no evidence of intoxication. Recovery was immediate, showing complete destruction of poison.

(5) Duodenal fluid (X), 20 c.c., plus both lungs ground to a thin paste with sand, water, chloroform, and toluol. Autolysis for sixty-six hours, heated at 60° C. for one half hour, centrifugalized, and filtered, giving a clear, odorless, pale amber colored fluid. The entire filtrate, 125 c.c. in amount, was given intravenously to dog O-61 with but slight evidence of intoxication.

(1) *Dog O-64.*—Male pup; weight 10¼ pounds.

Mar. 27, 12.30 P. M. Intravenous injection of a fluid mixture of immune dog's mucosa digested with duodenal loop fluid. Autolysis for five days and preparation for injection in the usual manner. This caused great and prolonged drop in blood pressure with the appearance of diarrhea within thirty minutes. 4 P. M. Dog is cool and badly shocked. Much fluid feces. 10 P. M. Dog is moribund and cool.

Mar. 28, 9 A. M. Autopsy shows typical picture of acute poisoning with duodenal loop fluid.

(2) *Dog O-63.*—Adult fox-terrier, male; weight 10 pounds.

Mar. 27, 12 M. Intravenous injection of fluid mixture of spleen emulsion from immune dog plus duodenal fluid (X), 20 c.c. Autolysis for three days and preparation as usual. This clear broth, 75 c.c. in amount, gave only a slight drop in blood pressure (kymograph). 4 P. M. One semifluid stool; no vomiting. No evidence of intoxication.

Mar. 28. Dog looks rather sick. No diarrhea. 1 P. M. Dog is quite sick; etherized.

Autopsy.—Extensive bronchopneumonia with hemorrhage and edema of both lungs. Liver shows areas of necrosis and hemorrhage. There is only a slight congestion of the intestinal mucosa. It is unlikely that much of the intoxication was due to the duodenal loop poison. Death was probably due in great measure

to the acute pneumonia associated with aspiration of vomitus at the time of anesthesia.

(3) *Dog O-56.*—Fox-terrier, male; weight 14 pounds.

Mar. 22, 4 P. M. Intravenous injection of a fresh mixture of duodenal loop fluid (X), 15 c.c., plus fresh Buchner press liver juice, 50 c.c., of immune dog. Kymograph observation and ether anesthesia. This caused a marked drop in blood pressure with slow recovery. Marked polypnea. 5 P. M. Dog appears greatly shocked. 10 P. M. Found dead and rather cold. Much feces and vomitus below cage.

Mar. 23, 9 A. M. Autopsy showed typical evidence of acute poisoning with duodenal loop fluid. The splanchnic engorgement and congestion are very intense.

(4) *Dog O-57.*—Small bulldog; weight 14½ pounds.

Mar. 24, 10.30 A. M. Intravenous injection of fluid mixture, duodenal loop fluid (X), 20 c.c., plus Buchner press liver juice of immune dog, 50 c.c. Mixture autolyzed for forty-four hours at 38° C., diluted, heated, and treated in the usual way for injection. This clear broth-like, odorless fluid gave a slight drop, followed by rapid return to normal blood pressure (kymograph). 12 M. No vomiting; slight diarrhea. Dog does not appear poisoned.

Mar. 25. Dog is normal.

(5) *Dog O-61.*—Small female; weight 11¾ pounds.

Mar. 25, 12 M. Intravenous injection of fluid mixture, duodenal loop fluid (X), 20 c.c., plus emulsion of lungs from immune dog O-51. Mixture incubated for sixty-six hours at 38° C. and treated in the usual way for injection. This clear fluid, 125 c.c., the total filtrate, caused little reaction on the part of the blood pressure. 2 P. M. Dog is sick, but only one stool is passed.

Mar. 26. Dog is well.

The preceding experiment (dog O–51) brings out interesting points. The dog was immunized by means of a mixture of a standard loop fluid digested with immune mucosa which had destroyed most of the poison; yet the dog developed a high grade of immunity as shown four days later when the animal survived a lethal dose of standard duodenal fluid.

Many of the immune organs were washed free from blood and ground to a paste which was combined with a lethal dose of the duodenal loop fluid, diluted with water to a thin paste, and allowed to autolyze at 38° C. for two to five days with chloroform and toluol. The filtrate was then tested on normal dogs by intravenous injection. The spleen and lung emulsion destroyed the poison. The liver juice (Buchner press), diluted and filtered, also destroyed it rapidly. The intestinal mucosa destroyed some of the poison but a part remained even after five days' digestion. A fresh mixture of liver juice and loop poison gave fatal intoxication, showing that

the reaction is not prompt or does not take place in the blood stream. This result serves as a control. If the protective action resides in a single type of cell, it is the endothelial cell that may be concerned, but it is of course possible that various body cells may develop the reaction or produce the ferment.

LIVER, SPLEEN, AND MUCOSA OF IMMUNE DOG DESTROY DUODENAL LOOP POISON.

Dog O-32.—Mongrel, male; weight twenty pounds.

Jan. 30. Duodenal fluid (dog O-12), 15 c.c., given intravenously. This caused vomiting, but no diarrhea.

Feb. 1. Duodenal fluid (dog S-51), 15 c.c., intravenously. This caused vomiting and dog appears somewhat poisoned.

Feb. 4. Dog appears fairly well; weight 18 pounds. Duodenal fluid (dog S-51), 20 c.c., injected intravenously. This caused vomiting but no diarrhea.

Feb. 11, 10 A. M. Duodenal fluid (dog O-26), 26 c.c., given intravenously. 11.30 A. M. Considerable vomitus, but no diarrhea.

Feb. 12. Dog appears quite sick. Temperature 34.2° C. Etherized and bled.

Autopsy.—Performed at once. The organs are practically normal. The mucosa of the small intestine is washed and scraped off carefully. The spleen is cut and ground up with sand and mixed with the mucosa.

The spleen-mucosa mixture was then added to 30 c.c. of duodenal loop fluid (dog O-26). This fluid had been tested and was known to be a lethal dose (compare dog O-31) for an animal of 13 pounds weight. This mixture, together with chloroform and toluol diluted to a thick emulsion by water, was incubated at 39° C. for three days. The mixture was then heated at 60° C. for one hour, centrifugalized, but not filtered. The turbid, rather soupy fluid, 100 c.c. in all, was given intravenously to dog O-42, a small female fox-terrier, weighing 11½ pounds. The injection caused a great drop in blood pressure with rapid return to normal after each injection.

Feb. 15, 2.30 P. M. Kymograph. 4 P. M. Dog seems quite well.

Feb. 16. Dog is normal.

The liver obtained from the immune dog O-32, normal in gross, weight about 300 gm., was minced and ground up with sand and combined with duodenal loop fluid (dog O-26), 32 c.c. This was a known lethal dose of loop fluid (dog O-31). The mixture was diluted suitably with 0.9 per cent. salt solution, and then chloroform and toluol were added. This mixture of immune liver tissue and duodenal fluid incubated for twenty-four hours at 40° C. was then centrifugalized, without heating, giving a cherry red, rather turbid fluid. The total amount, 85 c.c., given intravenously had a powerful depressor action (dog O-40, strong female pup; weight 21 pounds).

Feb. 13, 4 P. M. Kymograph. The blood pressure curve returned rapidly to normal and there was no slowing of the pulse. There was remarkable and forcible dyspnea. 6 P. M. Dog appears to be recovering normally from ether. No diarrhea.

Feb. 14. Dog normal in every respect.

The liver residue obtained after centrifugalization and removal of fluid tested above (dog O-40) was carefully saved and digested at 38° C. together with normal pancreas for a period of three weeks. This gave a clear, deep brown

fluid, strongly acid in reaction. It is made faintly alkaline by sodium hydroxide. The fluid was given rapidly intravenously to dog O-45, a small male fox-terrier, weighing 13½ pounds.

Mar. 6, 10 A. M. Ether anesthesia and kymograph. Intravenous injection of total fluid obtained, 270 c.c. This caused a rapid drop in blood pressure with return to normal at the end of thirty minutes. 12 M. No vomiting or diarrhea. Dog is recovering rapidly. There was no abnormality except prolonged oozing from the neck wound, probably associated with antithrombin excess in the blood.

Mar. 7. Dog is normal.

LIVER EXTRACT FILTRATE OF IMMUNE DOG DESTROYS DUODENAL LOOP POISON.

Dog O-55.—Mongrel pup, male; weight 20½ pounds.

Mar. 20, 4 P. M. Intravenous injection of extract made from the mucosa of the drained duodenal loops of dogs O-21 and O-24. The total amount, 125 c.c., caused no immediate change in blood pressure. 8 P. M. Much vomiting and bloody diarrhea. Pulse fair, but animal appears considerably shocked.

Mar. 21. Dog is still quite sick.

Mar. 24. Dog appears well. 12 M. Intravenous injection of 20 c.c. of duodenal loop fluid (dogs O-33 and O-34). This fluid had been standardized on normal dogs. 3 P. M. Dog is quite sick. There is marked salivation and vomiting with profuse diarrhea and severe shock. 5 P. M. Mucous diarrhea and severe intoxication.

Mar. 25. Dog is improving, but still weak.

Mar. 29. Dog in fairly good condition except for infected neck. Ether anesthesia, bleeding from carotid, and washing of organs free from blood with large amounts of normal saline given intravenously during bleeding.

Liver quite bloodless, weight 294 gm., minced and ground up with sand to a thick paste, which was placed in a Buchner press. About 50 c.c. of thick, viscid juice was expressed, and this was diluted to about 130 c.c. This thin, soupy material was allowed to undergo autolysis at 38° C. for sixteen days, giving a clear, deep brown fluid which filtered with ease.

30 c.c. of this clear filtrate were added to 20 c.c. of duodenal fluid (X), a known lethal dose. Autolysis of the mixture for three days gave a very slight turbidity. The material was centrifugalized but not heated, filtered, and injected intravenously, 50 c.c. in amount, into dog O-72, a strong male fox-terrier, weighing about 12 pounds. Kymograph record and ether anesthesia during the period of injection. The filtrate caused a drop in blood pressure followed by rapid recovery. There was no evidence of intoxication, no vomiting, and no diarrhea. The following day the dog was normal in all respects.

The same liver extract (dog O-55), 90 c.c. in amount, was kept in the incubator (38° C.) with chloroform and toluol for a period of sixteen weeks. At the end of this time filtration gave the same clear, whiskey-colored, odorless fluid. This was combined with fresh duodenal fluid, 50 c.c. (dog O-86). This mixture of immune liver juice and duodenal fluid was allowed to remain in the incubator with chloroform and toluol for ten days. At the end of this time the solution filtered with great rapidity. There was only the slightest turbidity, and no odor.

July 31, 12 M. Unheated fluid, 135 c.c., given intravenously to dog O-116, a young female mongrel, weighing 17½ pounds. 3 P. M. Dog greatly shocked. 4 P. M. Death, preceded by vomiting and diarrhea.

Autopsy.—Aug. 1. The usual picture following acute intoxication is present. This shows that the ferment capable of destroying the duodenal poison is an unstable body and may disappear from the immune organ extracts after a period of many weeks.

The preceding experiment (dog O–55) adds another important point. The immune liver juice kept in an incubator undergoes autolysis rapidly yielding a clear, whiskey-colored filtrate, which at the end of two weeks contains a ferment capable of rapidly destroying *in vitro* the duodenal loop poison. However, after a period of incubation at 38° C. for sixteen weeks the active body disappeared and the liver extract was inert towards duodenal fluid. This fact should be compared with the observations above (dog O–43), where it is noted that the immunity of an animal will vanish or drop nearly to a normal level during a period of four months.

DISCUSSION.

The series of experiments here reported provide, we believe, conclusive evidence that a definite intoxication occurs in animals with closed loops, and that the intoxication is due to absorption of the loop poison. There are good reasons for believing that the intoxication seen in cats and dogs with intestinal loops and in human intestinal obstruction is due to the same poison or a similar one. It is noteworthy that a dog recovering from simple obstruction showed little evidence of intoxication when a closed duodenal loop was established. Other evidence will be brought forward later to show that dogs with simple intestinal obstruction begin to acquire a certain immunity to the intoxication which will protect them against a closed-loop intoxication or the intravenous injection of duodenal loop fluid.

In a recent paper Hartwell (5) criticizes our conclusions and endeavors to minimize the factor of intoxication while emphasizing the loss of fluid as the essential and primary condition. In the conclusions of their first paper Hartwell and Hoguet (6) state: "The above findings indicate that death from intestinal obstruction in dogs results from the presence of toxic substances in the circulating

blood which produce fatal lesions in the kidney, liver and other tissues." In their second paper (7) they say: "The important element, therefore, in the development of the symptoms seen in intestinal obstruction in dogs is the loss of water due to vomiting. The symptoms of intoxication are those resulting from tissue disintegration following this loss."

We have claimed that intoxication is the primary feature and that loss of fluid is secondary to the intoxication, instead of the reverse, as claimed by Hartwell. In his last paper Hartwell (5) admits that there may be intoxication, and so reverts in part to the theory supported by his first paper. He states that intoxication can only appear when there is obvious injury to the intestinal mucosa, and in this we believe he is mistaken, although we admit that given an obvious mucosal injury with ulceration, the intoxication may be more profound. If the theory of injury to the mucosa is the essential factor, why do not all cases of gastro-enterostomy show signs of acute intoxication? Why do not dogs with drained loops where the gut is cut across and drawn into the abdominal wound die sooner than dogs with simple closed loops? Dogs with closed loops may show evidences of grave intoxication at the end of twenty-four hours and yet autopsy may show a collapsed loop with intact mucosa, containing a little pasty material. The entire intestinal mucosa shows very slight congestion, and it has been shown clearly that the poison concerned causes splanchnic dilatation and even paralysis. The mucosa of the loop is often slightly injected and Hartwell states that this indicates injury to the mucosa; however, the microscope shows a normal mucosa except for a slight widening of the capillaries in the villi.

We have held that the poison is elaborated by the mucosa, for when the mucosa is destroyed no poison is formed. It is conceivable that the mucosa can be disturbed so as to functionate abnormally, without any gross evidence of disturbance, and we may assume a perturbed physiological balance of the mucosa by which abnormal products are formed and secreted into the blood stream. We feel that the experiments here published give adequate basis for the belief that intoxication is the essential factor in intestinal obstruction or in the closed duodenal loop complex, and that the intoxica-

tion in the two conditions is similar if not identical, and the perverted activity of the mucosa, not an anatomical injury, is responsible for the formation of the poison.

It is clear that the cells and organ extracts of immunized animals can destroy the duodenal loop poison which is so resistant to simple digestion. The destruction *in vitro* during autolysis is quite rapid and the resultant material is non-toxic. The possibility that this immune tissue juice might be potent when given intravenously at once suggests itself. It is of course possible that the immune organ extract may destroy the poison *in vivo* and help to tide the animal over a period of acute intoxication. This point is being investigated at present.

CONCLUSIONS.

Dogs may be immunized against lethal doses of the duodenal loop poison by means of small doses of the loop fluid from dog or cat and by material obtained from human cases of intestinal obstruction. The immunity is transient and may disappear within a few weeks.

Dogs immunized by repeated doses of loop fluid show a definite resistance against the intoxication of a closed duodenal loop and may survive twice the usual period.

A dog that recovers from simple intestinal obstruction may possess a strong resistance to the intoxication of a closed duodenal loop, thus indicating a similar type of intoxication in the two conditions.

The sera of immune dogs are inactive when incubated with duodenal loop fluid.

The organ extracts and emulsions (liver, spleen, lung) of immune dogs rapidly destroy the loop poison during incubation *in vitro*.

This destructive property is possessed by a clear filtrate of the digested immune organs, excluding adsorption, and is lost after long periods of incubation (twelve weeks).

We are investigating the action of this immune organ extract to determine whether it can destroy the closed-loop poison *in vivo* and perhaps be of value in treatment.

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INTESTINAL OBSTRUCTION.

IV. The Mechanism of Absorption from the Mucosa of Closed Duodenal Loops.*

By G. H. WHIPPLE, M.D., H. B. STONE, M.D., and B. M. BERNHEIM, M.D.

(*From the Hunterian Laboratory of Experimental Pathology, Johns Hopkins Medical School, Baltimore.*)

In an earlier paper (1) we have shown that the poison of closed duodenal loops, which is responsible for the intoxication in that condition, is not absorbed by the mucosa of the unobstructed gut. We also tested the absorption of the same toxic fluid when introduced into recently isolated duodenal loops, and found that the dogs whose closed loops contain a double lethal dose of duodenal loop poison show no more intoxication than control dogs with empty closed loops, and die in about the same period, from which it is concluded that absorption from the lumen of the closed loop may be a secondary factor of intoxication and the important fraction is absorbed from the mucosa and not from the lumen of the loop. The observation of drained-loop dogs showing fatal intoxication confirms the conclusion that the greater part of the poison is absorbed from the mucosa itself, regardless of the amount of toxic material present in the lumen.

Similar experiments made by Davis (2) in this laboratory show that a closed duodenal loop is just as toxic when left washed and empty at operation or distended with a lethal dose of duodenal loop fluid from another animal. Davis also showed that repeated washing out of the stomach and duodenum, after ligating the bile and pancreatic ducts and establishing a low duodenal fistula, yields a certain amount of the toxic substance, a fact which may be interpreted to mean that the substance is excreted normally by the duodenal mucosa; and yet such a statement may exceed the facts. It

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has been shown by the writers that the toxic body is not present in demonstrable amounts in normal digested mucosa, but this does not preclude the possibility of its normal excretion in small amounts by the duodenum. It may be objected that the animal in the above experiment is not normal in that the bile and pancreatic juice are absent, the mucosa is constantly washed by water, and the animal is suffering from a certain amount of shock following extensive operative manipulations. The weight of evidence, however, seems to favor the possibility that the toxic substance may be excreted in small amounts by the normal mucous membrane of the small intestine. The formation of this toxic substance under abnormal conditions is rapid and abundant, as it can be demonstrated easily in the small amount of mucosa found in a closed or drained duodenal loop.

The essential point is, after all, the secretion of the toxic substance into the blood, which does not happen normally, but does occur in closed or drained duodenal loops and in intestinal obstruction. The important questions then are: What causes this changed function of the mucosa? Why does it form this poison and allow its passage into the blood? How does the mucosa readjust its equilibrium, and what may bring this about?

EXPERIMENTAL OBSERVATIONS.

CLOSED DUODENAL LOOP FILLED WITH TOXIC LOOP FLUID AT OPERATION. KILLED AFTER FOUR DAYS.

Dog O-100.—Young adult, male; weight 19 pounds.

July 16, 2 P. M. Ether anesthesia and operation. Closed duodenal loop with gastro-enterostomy made as usual. At the end of the operation the closed duodenal loop was filled with duodenal loop fluid, 50 c.c., obtained from dog O-86. This fluid had been standardized and 50 c.c. was fatal to a normal dog weighing 17½ pounds. 5 P. M. Dog recovering from operation; walks about and seems normal. 8 P. M. Some vomiting and diarrhea.

July 17. Dog appears like the usual closed-loop dog, and remains curled up quietly. Temperature 38.6° C. Weight 18¼ pounds. 5 P. M. Dog appears better. Temperature 38.6° C.

July 18, 10 A. M. Dog appears fairly well. Pulse is fair. Temperature 38.5° C. Weight 17½ pounds.

July 19, 9 A. M. Condition remains the same. Pulse rather weak. Temperature 38.4° C. Weight 17¼ pounds.

July 20, 11 A. M. Temperature 38.4° C. Weight 16¾ pounds. Pulse is fair and dog does not seem gravely intoxicated. Etherized and killed.

Autopsy.—Performed at once. Thorax, heart, and lungs normal. The peritoneum shows beginning purulent peritonitis with about 5 c.c. of thick, fibrino-purulent exudate in each flank. The surface is moist, injected, and shows fine grains of fibrin. The loop is distended tightly. The omentum is adherent about it. There are purplish patches showing through the wall. The ends are tightly closed and it contains 80 c.c. of thin slaty fluid having a very offensive odor. The mucosa of the loop is swollen and pinkish. There are purplish areas of inflammation with submucous hemorrhage and some superficial ulcers with obvious necrosis of the mucosa. Intestines and abdominal viscera are negative except for congestion. Liver shows cloudy swelling.

CLOSED LOOP DISTENDED WITH TOXIC FLUID AT OPERATION. KILLED AFTER FIVE DAYS.

Dog O-111.—Strong, white bulldog, male; weight 32 pounds.

July 24, 3 P. M. Ether anesthesia and operation. Closed duodenal loop with gastro-enterostomy was done as usual. At the end of the operation the closed duodenal loop was filled with 100 c.c. of duodenal loop fluid. The loop in this instance was somewhat longer than normally, but this amount of fluid distended it tightly. This fluid had undergone autolysis for weeks and filtered readily. 50 c.c. of the fluid was a lethal dose for a normal dog 20 pounds in weight.

July 25, 10 A. M. Temperature 39.1° C. Weight 31¼ pounds. Dog appears intoxicated to about the usual degree noted in control dogs with simple closed loops.

July 26, 10 A. M. Temperature 39.2° C. Weight 29½ pounds. Dog in fair condition and looks exactly like control dog. There is no evidence of increased intoxication from the fluid introduced into the loop.

July 27, 11 A. M. Dog has been vomiting a good deal. Temperature 38.8° C. Weight 28 pounds.

July 28, 10 A. M. Condition about the same. Temperature 39.2° C. Weight 27½ pounds.

July 29, 10 A. M. Dog looks very weak and will probably succumb during the day. Temperature 39.2° C. Weight 26½ pounds. Etherized and killed.

Autopsy.—Thorax, lungs, heart, and kidneys are normal. Liver is pale and shows evidence of fatty degeneration. The loop contains a good deal of fluid and is dilated with thickened walls. The ligatures at both ends have cut nearly through the wall, allowing the escape of fluid and entrance of a small amount of bile-stained material from the stomach. The mucosa is intact everywhere except at the site of ligation. There is a slight congestion of the mucosa of the loop and of the jejunum. It is possible that with the cutting through of these ligatures and beginning of the flow of normal secretion through the loop the intoxication in this animal would be overcome and recovery would take place, but it is obvious that the introduction of a large amount of duodenal loop fluid did not intensify the intoxication noted in the first few days when the loop was closed tightly.

CLOSED DUODENAL LOOP DISTENDED WITH TOXIC FLUID. RECOVERY.

Dog O-112.—Strong bulldog, female; weight 24 pounds.

July 24, 4 P. M. Ether anesthesia and operation. Closed duodenal loop with gastro-enterostomy formed as usual. At the end of the operation the closed loop

was distended with 75 c.c. of duodenal loop fluid obtained from dog O-95. This fluid had undergone autolysis for three days, followed by filtration but not heating. 60 c.c. had poisoned rapidly a normal dog weighing 13 pounds, causing death in two hours.

July 25, 10 A. M. Temperature 38.9° C. Weight 22¾ pounds. Pulse is fair and dog curled up quietly.

July 26, 11 A. M. Condition about the same. Temperature 37.8° C. Weight 21½ pounds.

July 27, 11 A. M. Dog looks much better, and is rather active. Temperature 38.1° C. Weight 21¾ pounds.

July 28, 10 A. M. Dog is fairly well. Pulse is of good tension. Temperature 38.8° C. Weight 21¾ pounds.

July 29, 10 A. M. Dog is rather sick, but not much shocked. Temperature 38.9° C. Weight 21½ pounds.

July 30, 10 A. M. Dog is much better. Temperature 38.3° C. Weight 23 pounds. Dog does not vomit and is eating food. It is evident that the ligatures have cut through and the intoxication is diminishing. Condition improved until Aug. 5, when the dog is etherized and killed. Weight 23½ pounds.

Autopsy.—Thoracic and abdominal organs normal. The loop shows both ligatures cut through, lying embedded in the mucosa on one side of the lumen. The lumen of the lower end is dilated to almost normal, whereas the upper end still shows some constriction. The wall of the loop is thickened and is somewhat dilated. The mucosa everywhere except at the site of ligation is intact and normal.

The three preceding experiments (dogs O–100, O–111, and O–112) indicate that the presence of large amounts of toxic, standardized duodenal loop fluid in a recently established closed loop will not modify the picture of intoxication. The control dog with an empty closed loop appears to be just as much intoxicated during the first forty-eight hours following the operation as the dogs whose closed loop is distended to its maximum capacity with old or fresh loop fluid.

The distended loops may show an early cutting through of the ligatures, with return to normal. We believe that pressure and distension are important factors in this result. A similar reaction is seen in the common bile duct when a ligature producing icterus cuts through in about five days and allows the free flow of bile, the ligature lying in the side of the duct wall. In the empty closed loop the fluid accumulates slowly and may be considerable after twenty-four to forty-eight hours, whereupon there will be violent peristalsis and a tendency for the ligatures at the ends of the loop to cut through the wall. Perforation or fatal intoxication almost always

supervene before this has taken place. But with a distended loop at the start, there will be violent peristalsis with increased tension on the end ligatures. This causes more or less pressure necrosis in the tissues below the ligatures, and the ligatures slowly cut and press into the tissue while the wall adheres behind the ligature and obliterates its track. Finally the ligature cuts through the soft mucosa and lies in the lumen adherent to a segment of the mucosa which is not cut through. The linear cut or ulcer of the mucosa heals over rapidly and the gut slowly comes back to its normal diameter and thickness. Shortly after a free flow through the loop is established the toxic symptoms vanish and the dog begins to eat and put on weight.

DRAINED DUODENAL LOOP. DEATH IN FIVE DAYS.

Dog O-21.—Black mongrel, male; weight 23½ pounds.

Jan. 8. Ether anesthesia and operation. Drained loop made as usual with a large drainage tube sewed into one end. Gastro-enterostomy.

Jan. 9. Good drainage from tube and loop washed out with water. Dog is in good condition and eats.

Jan. 10. Dog is active. Drainage has taken place and loop is washed out again with water.

Jan. 11. Dog seems well. Drainage tube has come out of the loop, but the loop is washed as usual.

Jan. 12. Evidence of distemper. The skin on the abdomen shows digestion and bile staining.

Jan. 13. Found dead.

Autopsy.—The ligature below the pancreatic duct had cut through, allowing bile and pancreatic juice in small amounts to escape into the loop. The other ligature is tight. The loop contains 1 to 2 c.c. of thick buttery material. Its mucosa is reddened, but everywhere normal except for congestion. The small intestine shows a diffuse injection with a red velvety mucosa and contains abundant fluid feces. The duodenum above the ligature shows a congested, reddish purple, velvety mucosa. The gastro-enterostomy is normal. Stomach shows congestion in the cardiac portion. The peritoneum is everywhere clean, but the mesenteric vessels are injected and widely distended. Spleen and liver are definitely engorged and tense. Thorax, heart, and lungs are normal. There is evidence in this case that the characteristic toxic agent was present, causing fatal intoxication associated with a great deal of splanchnic congestion. The escape of pancreatic juice and bile in small amounts did not modify the reaction in the loop.

DRAINED DUODENAL LOOP. DEATH IN SIX DAYS.

Dog O-2.—Weight 17¾ pounds.

Oct. 16. Ether anesthesia, operation, and production of a drained duodenal loop, extending externally through the abdominal wound. Gastro-enterostomy.

Oct. 22. Loop had been rinsed out with water daily by inserting a catheter and using a liter or more of fluid. 9 A. M. Dog found dead.

Autopsy.—The peritoneal cavity is clean. The duodenal loop is normal and the lower end firmly fixed by granulation in the abdominal wound. The loop is collapsed and contains about 2 c.c. of pasty material. The mucosa close to the opening exteriorly shows a small ulcer involving the mucosa, measuring only 6 mm. in length. Elsewhere the mucosa is normal and intact, having a pale, pinkish mottled color. The other abdominal viscera are normal. The heart contains blood clots. The lungs show a few small purplish patches of consolidation not over 0.5 cm. in diameter.

DRAINED DUODENAL LOOP. DEATH IN TWENTY-FOUR HOURS.

Dog O-24.—Strong mongrel, male; weight 20 pounds.

Jan. 15, 3 P. M. Ether anesthesia and operation as usual. Drained duodenal loop of the usual length isolated, but both ends drained through the abdominal wound and drainage tubes sewed into the open ends. Gastro-enterostomy.

Jan. 16, 3 P. M. Dog appears very much intoxicated. Temperature is subnormal. Loop washed out thoroughly with warm water. 4 P. M. Death.

Autopsy.—Performed at once. The peritoneum is clean. Thorax is normal. The blood is fluid and clotted slowly only when in contact with the body tissues, indicating the presence of antithrombin. The spleen and liver are moderately congested. The jejunum shows a mottled mucosa, reddish areas alternating with paler pink areas. The loop shows a good deal of edema and hemorrhage in the distal portions which were sewed into the abdominal wall. This was probably due to constriction and interference with the blood supply. Elsewhere the loop mucosa shows a moderate grade of congestion and is intact. It is possible that a part of this acute intoxication was due to the obvious interference with the blood supply to the ends of the drained loop.

DRAINED DUODENAL LOOP. DEATH IN THREE DAYS.

Dog O-36.—Large mongrel, male; weight 30½ pounds.

Feb. 5, 4 P. M. Ether anesthesia and operation. Drained loop made as usual and gastro-enterostomy established. Tubes sewed in both ends. One was brought out through a puncture wound in the right rectus, and the other through the abdominal wound.

Feb. 6, 10 A. M. Dog shows considerable drainage from the duodenal loop which is washed out thoroughly with water and a very dilute solution of permanganate. Temperature 37.7° C. 4 P. M. Dog appears intoxicated. Temperature 38.9° C.

Feb. 7, 10 A. M. Temperature 38.5° C. Dog looks better and loop is washed out thoroughly with a large amount of dilute permanganate solution. 2 P. M. Dog appears gravely intoxicated.

Feb. 8, 12 M. Much vomiting. Death.

Autopsy.—Performed at once. The peritoneum is clean. The stomach is full of bile-stained fluid similar to that found in intestinal obstruction. The intestine everywhere is full of a similar fluid. The mucosa of the small intestine has a patchy, injected appearance. Gastro-enterostomy is clean. Drainage tube ex-

tended well into the loop, and the mucosa in contact with the tubes is red and swollen, and there is evidence of some inflammation. There is some mucus and fluid accumulated in the dependent portion of the loop. Between the ligature just below the pancreatic duct and the upper drainage tube is a portion of intestine about two inches in length which was not drained and in which material had accumulated. Other organs are negative except for a few very small patches of hemorrhagic bronchopneumonia in one lung.

The loop showed incomplete drainage in spite of large amounts of wash fluid (permanganate), because a small bit of intestine between the upper tube and the ligature below the pancreatic duct did not open freely into the loop, the drainage tube being too large and long. The accumulation of the characteristic fluid in this small portion of the duodenum may have hastened the fatal intoxication which was acute and characteristic.

The group of dogs (O–21, O–2, O–24, and O–36) with drained and washed loops shows that intoxication may develop under such conditions, and the only possible source for the poison is the mucosa. Moreover, the toxin can be demonstrated in the mucosa of the loop and in the loop washings. In an earlier publication (3) similar cases were cited and we have now a series of experiments that prove this point beyond doubt. It will be recalled that under favorable conditions a dog with a drained loop may live in good health for many weeks, and it may now be assumed that such a dog has developed a strong immunity that protects it against the intoxication. An experiment cited below gives evidence of an immunity demonstrable in the mucosa of a drained-loop dog. The immune mucosa can destroy a lethal dose of duodenal loop fluid after incubation for a few days, while normal mucosa has no such activity.

Conditions do not seem to be as abnormal in a drained loop as in a closed loop where we may have accumulation of much toxic fluid under tension with enormous multiplication of bacteria and often some injury and ulceration of the loop wall. The drained loop is rinsed out carefully each day with much fluid, and little or no material accumulates. Yet the intoxication develops and is usually fatal in the majority of cases. We can eliminate great numbers of bacteria by the repeated washings, although, of course, bacteria are still present but probably in less numbers than in the normal gut. It is clear that the bacterial flora will be much modified in a drained loop even if not greatly lessened in amount. The normal flow of the products of digestion and excretion is obliterated and

we are inclined toward the view that it is this essential deviation from normal which causes the perverted activity of the mucosa.

DUODENAL LOOP DRAINING INTO THE JEJUNUM. INTOXICATION AND IMMUNITY.

Dog O-41.—Strong bulldog, male; weight 25 pounds.

Feb. 14, 4 P. M. Ether anesthesia and operation. Gastro-enterostomy as usual. The duodenum just below the pancreatic duct is cut across and ligated with inversion of ends and closure. This partially closed duodenal loop can drain freely into the jejunum.

Feb. 15, 10 A. M. Dog looks well. 4 P. M. Dog appears toxic, but there is no vomiting.

Feb. 16–19. Dog appears well.

Mar. 1. Dog is losing weight but appetite is good; weight 21 pounds.

Mar. 7. Animal continues to lose weight. Temperature 38.9° C. Weight 18 pounds. There is some vomiting. 4 P. M. Dog killed.

Autopsy.—Beginning peritonitis with abscesses forming about the duodenal section. Thorax, heart, and lungs normal. Spleen and liver swollen and grayish. Duodenal loop is empty except for a little mucus. Other organs are relatively negative. The mucosa from the duodenum, jejunum, and ileum are washed, scraped off carefully, and combined with the duodenal loop fluid (dog O-33), 30 c.c. in amount. The fluid had been standardized and shown to be toxic, 25 c.c. poisoning fatally in four hours a normal dog (No. O-46), weighing 16 pounds.

The mixture of mucosa (dog O-41) and duodenal loop fluid (dog O-33) was allowed to undergo autolysis at 38° C. for eight days, and was then heated at 60° C. for one hour, centrifugalized, and filtered. This gave a clear, odorless broth, 115 c.c. in amount, which was injected intravenously into a normal dog (No. O-51), weighing 15½ pounds. It caused an initial drop in blood pressure followed by a rise (kymograph) with return to normal in thirty minutes. After six hours the dog appeared fairly well. Pulse good. There has been no vomiting and only one soft stool. The next day the dog is normal in every way. The experiment indicates that this loop dog (No. O-41) had been immunized by the presence of his partially drained loop, causing more or less intoxication. The immune mucosa *in vitro* completely destroyed with considerable rapidity a standard toxic dose of duodenal loop fluid.

DUODENAL LOOP DRAINING INTO THE JEJUNUM. DEATH IN FOUR DAYS.

Dog O-19.—Active young female; weight 17 pounds.

Dec. 14, 3 P. M. Ether anesthesia and operation. Gastro-enterostomy was done at the usual place and a single ligature placed at the site of the usual upper ligature of the closed duodenal loop. The lower ligature was not placed, so that a part of the duodenum is isolated which can drain freely into the jejunum. The gastric contents, bile, and pancreatic juice escape through the gastro-enterostomy.

Dec. 15. Dog appears normal. Temperature 38.6° C.

Dec. 16. Temperature 37.9° C. Dog seems well.

Dec. 17. Temperature 38° C. Dog has lost weight. Weight 15 pounds. At this time the dog had a violent convulsion.

Dec. 18. Temperature 36.6° C. Dog appears sick. 3 P. M. Temperature 32.4° C. Animal desperately ill and died shortly after this observation.

Autopsy.—The peritoneum is normal. The partially closed loop contains about 10 c.c. of bile-stained, alkaline fluid. Its mucosa is normal, except for a slight congestion which is present as well in the jejunum. There is free communication between the duodenum and the upper portion of the jejunum. The small intestine shows a mottled, pink and red mucosa and contains a large amount of fluid fecal material. Stomach contains bile-stained fluid with alkaline reaction. Gastro-enterostomy is perfect. Liver, spleen, and kidneys show some congestion. Heart contains fluid blood. The lungs show small patches of hemorrhagic bronchopneumonia in the lower lobe.

DUODENAL LOOP DRAINING INTO THE JEJUNUM. INTOXICATION.

Dog O-20.—Mongrel bulldog, male; weight 22½ pounds.

Jan. 3, 3 P. M. Dog is in poor condition. Ether anesthesia and operation. A partially isolated duodenal loop, similar to that described in the preceding experiment, was made with gastro-enterostomy in the usual location.

A single ligature was placed just below the pancreatic duct, completely occluding the lumen of the duodenum.

Jan. 4, 4 P. M. Temperature 38.1° C. Dog is sick and has diarrhea.

Jan. 5, 10 A. M. Temperature 38.7° C. Diarrhea continues.

Jan. 6, 10 A. M. Temperature 38° C. Diarrhea still more marked. Dog is very quiet and refuses food.

Jan. 7. Found dead.

Autopsy.—General peritonitis with a good deal of purulent exudate due to leakage at the site of gastro-enterostomy. This is a recent peritonitis, and the symptoms of intoxication which preceded its development associated with diarrhea, etc., were probably due to the intoxication from the partially closed duodenal loop.

This group of experiments (dogs O–41, O–19, and O–20) brings out two points. Ligation or section across the duodenum below the pancreatic duct together with a gastro-enterostomy just beyond the duodenojejunal junction produces a condition which we may call a partially or half closed loop. This half closed loop drains freely into the upper jejunum, and can be washed incompletely by a back flow of fluids, passing from the stomach through the gastro-enterostomy. The presence of such a partially isolated duodenal loop may be associated with intoxication, more or less severe, which will bring about an immunity reaction in the body cells. The intestinal mucosa from such a dog has the characteristic property of immune tissue; it can destroy with some rapidity the duodenal loop fluid *in vitro* and render the mixture harmless when given intravenously to a normal dog. The normal mucosa does not possess this activity.

CLOSED DUODENAL LOOP PLUS BILE. DEATH ON SECOND DAY.

Dog O-10.—Fox-terrier, male; weight 19 pounds.

Nov. 6, 3 P. M. Ether anesthesia and operation. The pancreatic duct is isolated by ligature and cut. The lower ligature of the closed loop is placed in the usual situation. The upper ligature is placed between the bile duct and the pylorus. The common bile duct was left open and drained freely into the closed loop.

Nov. 7, 12 M. Dog appears sick. Temperature 36.9° C.

Nov. 8, 9 A. M. Dog found dead.

Autopsy.—Performed at once. Numerous fat necroses throughout the peritoneal cavity, indicating the escape of pancreatic juice at the site of operation. The duodenal loop had ruptured with the escape of bile-stained fluid and obvious peritonitis. The loop shows necrosis with ulceration of the mucosa and submucous hemorrhage and the mucosa is very soft and easily separated. The fluid is slimy, thick, and deeply bile-stained. The lungs show some edema and areas of purplish pneumonia. Other viscera are normal except for engorgement.

CLOSED DUODENAL LOOP PLUS BILE. DEATH IN TWENTY-FOUR HOURS.

Dog O-12.—Mongrel, female; weight 21½ pounds.

Nov. 21, 4 P. M. Ether anesthesia and operation. The isolated duodenal loop included about six inches of jejunum, being somewhat longer than usual. The upper ligature is placed between the bile papilla and the pylorus. Gastroenterostomy as usual. The pancreatic duct is ligated and cut.

Nov. 22, 9 A. M. Temperature 38.9° C. 1 P. M. Death.

Autopsy.—Performed shortly after death. The peritoneum contains a few cubic centimeters of blood-stained fluid with delicate grains of fibrin over the intestinal coils in the region of the liver. The pancreas shows a few fat necroses around the head of the organ. The loop is greatly dilated with fluid. Its wall is thin and shows subserous hemorrhages. The mucosa is intact, but obviously inflamed and swollen and scrapes off easily. The liver and spleen show a good deal of congestion. The small intestine shows a reddish congested mucosa. The other organs are negative. The duodenal loop fluid in this case was tested on a normal dog (No. O-28), and 70 c.c. given intravenously caused rapid and fatal intoxication, with death in two and one half hours. Weight 11½ pounds.

CLOSED DUODENAL LOOP PLUS PANCREATIC JUICE. DEATH ON FOURTH DAY.

Dog O-6.—Black mongrel, male; weight 19 pounds.

Oct. 30, 3 P. M. Ether anesthesia and operation. Duodenal loop made with the lower ligature in the usual situation. The upper ligature is placed above the pancreatic duct, which opens freely into the closed loop. The common bile duct is isolated between the ligatures and cut.

Oct. 31. Dog does not appear intoxicated to a grave degree and takes a little food.

Nov. 1. Dog seems fairly well. Temperature 38.1° C.

Nov. 2, 9 A. M. Found dead, but quite warm.

Autopsy.—The peritoneal cavity is filled with turbid, yellow, bile-stained fluid.

Thorax negative except for a few patches of bronchopneumonia. Spleen and liver slightly congested. The loop shows a rupture in the middle third where the wall shows extensive ulceration and softening. On cutting open the loop the ends are found to be tightly closed and the mucosa shows extensive ulceration and necrosis with submucous hemorrhage and a great deal of ecchymotic reaction, edema, and exudation. The small intestine shows a purple, mottled mucosa with evident engorgement of the mesenteric vessels.

CLOSED DUODENAL LOOP PLUS PANCREATIC JUICE. DEATH ON THIRD DAY.

Dog O-7.—Black and tan mongrel, male; weight 15 pounds.

Oct. 31, 4 P. M. Ether anesthesia and operation. The closed duodenal loop is made as in the preceding experiment, the lower ligature being in the usual location, but the upper ligature well above the bile and pancreatic papillæ. The common bile duct is doubly ligated and cut.

Nov. 1, 9 A. M. Dog curled up quietly. Temperature 38.9° C.

Nov. 2, 9 A. M. Death.

Autopsy.—Performed at once. The peritoneum contains about 100 c.c. of blood-stained, turbid fluid. The serous surfaces are specked with small hemorrhages, and the reaction is like that following the injection of duodenal fluid into the peritoneal cavity. The duodenal loop shows areas of hemorrhage, necrosis, and ulceration with perforation in the middle third. The mucosa everywhere is coated with mucus, deeply injected, and swollen, with extensive inflammatory reaction. There are deep ulcers with hemorrhages in the submucous tissue. The upper ligature is not quite tight and a little exchange of fluid may have been possible. Thorax. heart, and lungs negative. Spleen, liver, and kidneys are engorged. The jejunum shows a congested and velvety mucosa with deeper purplish patches. The lower portion of the small intestine is also congested. The picture is practically identical with that which may be found following intraperitoneal injection of heated, filtered duodenal loop fluid.

DUODENAL LOOP PLUS THE PYLORIC HALF OF THE STOMACH. DEATH ON FOURTH DAY.

Dog O-15.—Strong mongrel, male; weight 45 pounds.

Nov. 30, 2 P. M. Ether anesthesia and operation. Common bile duct and pancreatic duct ligated and cut. Stomach bisected. Gastro-enterostomy made by mistake into the pyloric half of the stomach, and the upper or cardiac portion completely isolated. The partially closed loop then contained the normal amount of duodenum plus the pyloric portion of the stomach which opened into the jejunum through a gastro-enterostomy.

Dec. 1. Condition as usual. Temperature 38.3° C.

Dec. 2, 3 P. M. Condition about the same. Temperature 38.8° C.

Dec. 3, 9 A. M. Temperature 37.5° C. There is some diarrhea and much vomiting. 12.30 P. M. Temperature 38.5° C. Dog seems very sick.

Dec. 4, 9 A. M. Found dead.

Autopsy.—There is some fresh peritonitis about the pyloric portion of the loop, and a few fat necroses around the head of the pancreas. The loop mucosa is intact and the duodenum contains about 100 c.c. of the usual brown soupy fluid. This material is shown to contain the usual toxic material. The lungs show a few patches of bronchopneumonia in the lower lobes.

CLOSED DUODENAL LOOP PLUS THE PYLORIC HALF OF THE STOMACH. DEATH ON THIRD DAY.

Dog O-17.—Large male; weight 43 pounds.

Dec. 7, 3 P. M. Ether anesthesia and operation. Stomach is bisected and gastro-enterostomy is made between the cardiac portion and the jejunum. Bile duct and pancreatic duct ligated and cut. The closed loop included the pyloric portion of the stomach with the entire duodenum and about three inches of the jejunum, with a simple ligature at the lower end of the loop.

Dec. 8, 10 A. M. Dog seems fairly well. Temperature 37.7° C.

Dec. 9, 9 A. M. Temperature 37.9° C. Dog is vomiting repeatedly.

Dec. 10, 9 A. M. Found dead, but quite warm.

Autopsy.—The peritoneal cavity contains blood-tinged, turbid fluid, about 150 c.c. There has been a rupture of the loop in the descending arm of the duodenum, where there are extensive areas of hemorrhage and ulceration. The pyloric portion of the stomach included in the loop is somewhat reddened but intact. The small intestine shows a congested velvety mucosa. The pancreas shows a few fat necroses around the head. The lungs are normal. The heart shows several adult specimens of *Filaria imitis.*

The preceding group of experiments shows that a closed duodenal loop is quite as toxic when the pancreatic juice or bile flows into it or when it includes the pyloric portion of the stomach. In the latter instance the dogs survived slightly longer, but this may be explained partly by the fact that the dogs in these experiments were unusually large and strong. These animals may survive a simple closed loop for a period of four or even five days.

DISCUSSION.

It will be recalled that a closed duodenal loop is isolated between two ligatures, and the continuity of the intestinal tract is established by means of a gastro-enterostomy. The intoxication under such conditions is very acute. If the lower ligature is omitted we form a partially closed loop which drains freely into the jejunum. Under the latter conditions the intoxication is less marked but obvious; immunity may result and be clearly demonstrated. With a simple obstruction of the upper jejunum a dog may live four to eight days, which is considerably longer than in the case of a simple closed loop. The intoxication is clearly of the same type; but why does the simple obstruction cause less acute symptoms?

Does the bile or pancreatic juice have the power of neutralizing or destroying the poison? This question is answered in the negative

by the last group of experiments. Moreover, the gastric juice is unable in any way to neutralize the poison formed in the loop or to lengthen the life of the animal. The drained duodenal loops give rise to the same type of intoxication, yet the lumen here is free from any fluid toxic accumulation. There is good evidence that the poison is not absorbed by the normal mucosa. Furthermore, absorption is not appreciably more rapid from a closed loop recently filled with the toxic fluid than from a simple closed loop.

This again emphasizes the fact that the mucosa is the source of the toxic absorption rather than the material accumulated in the lumen of the gut. The bile and pancreatic and gastric juices have no power to destroy the poison or check its production in a closed loop. Removal of the fluid by vomiting is not an essential feature, as the poison will not be absorbed from the lumen.

The essential feature perhaps is the flow of intestinal contents which provides the normal environment of the cells of the mucous membrane. When this flow is completely stopped, as in a closed loop, we note the most acute type of intoxication. It is probable that the abnormal fluid accumulation and perhaps bacterial growth may stimulate the mucosa to produce greater amounts of toxin. When simple obstruction is present there is an interchange of fluids, the current in the main being the reverse of normal, resulting in accumulation of fluid in the stomach and vomiting. Intoxication develops, but it is not as intense as in a closed loop, the reason for this being perhaps that fluids are still passing over the mucosa and tend to check the accumulation of the toxic substance within the mucosa which is most evident in the simple closed loops.

Our work with the toxic substance within the closed loops has brought out several points of interest bearing on its nature. It is clear that the poison is resistant to ordinary digestion or autolysis (pancreas or intestinal mucosa), and it can be boiled over a free flame, when in a non-coagulable fluid, without destruction. When the toxic substance is digested for weeks and months with normal intestinal mucosa it may be completely or partially destroyed. For this reason the duodenal loop fluid may deteriorate slowly, as in collection of the material it is easy to include a little of the mucosa. It can be shown that the mucosa of a closed loop, where the dog was

resistant and perhaps survived four days, can destroy the loop poison much more rapidly than normal mucosa. This fact must be kept in mind when making digestion extracts of the mucosa of closed loops, as prolonged digestion is apt to destroy some or all of the contained poison.

Normal organ extracts can destroy the duodenal loop poison when incubated for many weeks and the liver extract is most active, but their activity is far inferior to the same materials derived from immune dogs. When a sublethal dose of poison is introduced intravenously in a normal animal, it is probably fixed and destroyed by the tissue cells, and the capacity of destruction is greatly increased by this procedure, resulting subsequently in more or less immunity. The cell digestion and destruction of the poison is shown crudely by the incubation of mixtures of organ juices and loop poison. This suggests some ferment as the active factor.

Another point comes out clearly in our series of experiments. There seems to be a slight but definite seasonal fluctuation in the resistance of dogs to a duodenal loop, whether closed or drained. The operations have been done by the same persons and a great variety of animals have been included in the experiments. Dogs have a slightly greater resistance in the spring and summer than in the fall and winter. We believe that food, type of animal, and operative technique can be ruled out. The only possible factor is the temperature, and we believe that this is concerned in the slightly greater resistance of the dogs during the warm season.

The intoxication is associated with much loss of body heat and lowering of temperature. When the room temperature is between 80 and 90° F. it is obvious that there will be much less loss of body heat than with a room temperature of 60 to 70° F. This point is recognized in the treatment of operative shock, but perhaps is not sufficiently emphasized in connection with the treatment of intestinal obstruction and intoxication, which, when uncomplicated, may be associated with a subnormal temperature.

CONCLUSIONS.

Intoxication is evident in a drained duodenal loop whether it opens externally or into the jejunum and may be associated with

more or less immunity which can be demonstrated after a period of days.

Intoxication with a closed duodenal loop is identical whether the loop is left empty at operation or filled with a lethal dose of loop fluid. This again emphasizes the fact that absorption of the poison is essentially from the mucous membrane rather than from the contents of the closed loop.

The intoxication of a closed duodenal loop is not modified by the presence of bile, pancreatic juice, or gastric secretion.

Cessation of the normal flow of intestinal fluids which bathe the mucous membrane may be essentially responsible for the perverted activity of the mucosa and secretion of a poisonous material into the blood.

Animals may be slightly more resistant to closed or drained loops during the warm months, which may be explained by the increased loss of body heat in the colder months. This indicates that cases of acute intestinal intoxication with subnormal temperature may be benefited by a generous supply of artificial heat.

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STUDIES IN TISSUE SPECIFICITY.

II. The Ultimate Fate of Mammalian Tissue Implanted in the Chick Embryo.*

By JAMES B. MURPHY, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 33 and 34.

In a previous paper I have reported the fact that various mammalian tissues grew when implanted in the developing avian embryo, whereas in the adult chicken the same tissues were unable to survive.[1] It was later shown that the mammalian cells could be grown for long periods in the embryonic host if removed before hatching time and replanted in a young embryo.[2] That foreign tissue underwent no injurious change as a result of its long sojourn in the embryo was shown by the fact that growth continued when it was returned to its native species. If grafted into the adult fowl, or even into the newly hatched chick, the cells soon died and were absorbed. The quality that makes the chicken an unsuitable host for the growth of tissue of a foreign species seemed to develop some time between the eighteenth day of incubation and the first day of extra-shell life.

With the hope of throwing some light on the factor of resistance, a study has been made of the histological manifestations. The points considered are: first, the fate of an established and proliferating graft when left in the embryo during the development of the resistance; and, second, the fate of foreign tissue introduced during and after the onset of the refractory state.

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1 Murphy, J. B., *Jour. Am. Med. Assn.*, 1912, lix, 874.

2 Murphy, J. B., *Jour. Exper. Med.*, 1913, xvii, 482; *Proc. N. Y. Path. Soc.*, 1913, xii, 206.

The Fate of Tissue Implanted in the Adult of a Foreign Species.—The histologic changes about grafts from a foreign species introduced into the adult chicken closely resemble those observed about grafts of a chicken sarcoma in an immune animal.[3] In fact the histologic process which goes on when a living tissue is grafted into an animal unsuitable for its growth seems to have the same general characters, regardless of the source of the host's resistance. This is true in the case of animals naturally immune, either individually or because of variety or species; those with so called acquired immunity, traceable to recovery from an implanted tumor; and finally, in the case of those made artificially immune.[4]

To summarize the histological picture briefly, one finds about the strange graft an edema and reaction to the incidental trauma, followed by an increase in the fibroblasts in the surrounding host tissue, and then a budding out of the blood vessels and an infiltration of the tissues round about with small mononuclear cells. In birds, as Loeb and Addison have shown,[5] the accumulation of small round cells may be so marked that a considerable nodule of lymphoid tissue is formed (figure 1). The cells of the graft die more or less quickly, and in the meantime the connective tissue becomes more abundant, surrounding and invading the tissue remains, and separating the mononuclear cells into small islands. Finally the mononuclear cells disappear and the process subsides into a scar. There is a wide variation in the rapidity and extent of the reaction. This is especially noticeable when the same tissue is introduced into different species. Rat tissue in a mouse brings about a slow reaction, some of the rat cells surviving as long as nine days; and frequent mitotic figures are seen during the first few days. When, however, the rat tissue is grafted into a more distantly related species as the chicken, the rat cells are entirely disintegrated by the third day, and there is a rapid and extensive infiltration with small mononuclear cells. Some individual variation exists, but the fact holds true in

[3] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

[4] For the literature, see Da Fano, C., *Ztschr. f. Immunitätsforsch., Orig.*, 1910, v, 1.

[5] Loeb, L., and Addison, W. H. F., *Arch. f. Entwcklngsmchn. d. Organ.*, 1911, xxxii, 44.

general that the more distantly related the species, the more quickly the cells of the one will be disintegrated when introduced into the other.

The Fate of Foreign Tissue in the Newly Hatched Chick.—When grafts from a foreign species are introduced into the newly born chick the series of reactions are the same as those observed in the adult. The cells survive about the same length of time, and the reaction is observed to take place in the same sequence. Perhaps on the whole the round cell infiltration is not so extensive and the connective tissue reaction is more abundant, the process subsiding more quickly than in the adult animal.

The Fate of Established Grafts of Foreign Tissue when Left in the Embryo during the Development of the Resistant Condition.—The possibility was early considered that the cells of a foreign species, if left in the embryo during the supposed gradual development of the resistant factors,[6] might undergo an adaptation for growth in the adult. Such an adaption I find to be entirely absent. The refractory condition, instead of being of a gradual development, seems quickly to attain its full power. In order to study its time of onset and histological manifestations, grafts of rat sarcoma were inoculated into a number of chick embryos, long enough in advance of the refractory period to allow them to become established. The time found most suitable for the inoculations was from the 12th to the 15th day of incubation. The inoculated embryos were killed in lots of four or five at twenty-four hour intervals, beginning with the 17th day, and the grafts and their surroundings were examined histologically.

As late as the 18th day of incubation the grafts were found to be in active growth with no evidence of a defensive reaction on the part of the embryo (figure 2). On the 19th day most of the rat cells appeared to be still in good condition. Some of the cell nuclei, however, took the basic stain more deeply than normally and fewer mitotic figures were present. At the edge of the graft the connective tissue elements had begun to increase. By the 20th day the nuclei of most of the rat cells took a deep basic stain, and among the few cells retaining their normal staining reaction, a mitotic figure

[6] Murphy, J. B., *loc. cit.*

was rarely found. There was some necrosis in the centre of the graft. In a few cases there was a moderate amount of round cell infiltration. Some specimens showed a local accumulation of polymorphonuclear leucocytes. The uniform and striking change was in the connective tissue elements about the graft. By the 20th day they had increased markedly, forming a capsule of which strands had begun to invade the graft. Specimens, taken on the 21st and 22d day of incubation, showed a continuation of this connective tissue invasion and replacement (figure 3). On the 21st day only a few scattered rat cells could be identified in the mass of reactive tissue, and by the 22d day all of these had disappeared. At this late period small clumps of round cells were more numerous. The only traces of the graft were occasional fragments of necrotic tissue staining pink with eosin.

The Fate of Grafts of Foreign Tissue Implanted during and after the Onset of the Refractory Period in the Embryo.—The foregoing findings show that the refractory period comes after the 18th day of incubation. In order to study the reaction of the embryo to grafts introduced during and after the onset of resistance bits of rat tumor were inoculated on the 17th, 18th, and 19th days and were removed at twenty-four hour intervals. Examinations of these specimens gave uniform results with those observed in the preceding experiment. Grafts inoculated on the 17th day and removed on the 19th day of incubation showed an increase in the connective tissue about the graft, with perhaps an occasional group of small round cells. There was marked degeneration in the center of the graft. The cells at its edge showed an occasional mitotic figure but the nuclei of most of the rat cells took a deep basic stain. The graft itself was unvascularized although the blood vessels had increased in number around it. Many degenerate forms of tumor cells were present. The 20th day specimens showed an extension of the same process. There were fewer tumor cells with normal staining reaction, rarely a mitotic figure, and the connective tissue capsule was markedly increased, with beginning invasion and beginning organization of the graft remains (figure 4). The process had an end result resembling that seen in the fate of the organized graft. On grafts introduced as late as the 19th day some intact rat cells may be

seen up to the 22d day, but these were embedded in a thick mass of connective tissue. The round cell infiltration was more marked in these than in the other specimens.

DISCUSSION.

The results demonstrate that the period at which an established graft of foreign tissue in the chick embryo is checked in its growth corresponds in time with the period at which an introduced graft will no longer take. This suggests a radical change in the embryo which makes it an unsuitable host for the growth of the tissue of a foreign species. The period at which the refractory condition asserts itself is not remarkable for a sudden development or rapid growth of any important organ. The transfer of the respiratory function from the outer membrane to the lungs occurs only after the resistance has developed to a definite degree. This period from the 18th to the 21st day of incubation does not represent a period of more active growth of the embryo as a whole than do the earlier periods. It seems improbable, therefore, that the onset of the refractory condition can be correlated with a gross change in the embryo.

SUMMARY.

Rat tumors and other tissues of foreign species grow actively in the chick embryo until the onset of a refractory period. Grafts of rat sarcoma established and growing actively at the onset of this period show a rapid cessation of growth between the 18th and 19th days of incubation. This is followed by a widespread degeneration of the rat cells and a marked activity of the connective tissue elements in the embryonal tissue round about. An occasional specimen may show small mononuclear infiltration or rarely accumulations of polymorphonuclear cells in the neighborhood of the strange tissue. During the 20th and 21st days the connective tissue capsule increases rapidly, invading the graft and replacing it. The foreign cells disintegrate rapidly and by the 22d day have practically all disappeared. The period at which the established graft begins to degenerate, namely the 19th to the 20th day of incubation, is the one on which grafts of foreign tissue will no

longer take when implanted in the embryo. The cells at the edge of the graft of foreign tissue survive for a time. There is a rapid formation of a connective tissue capsule and an invasion of the grafts. The foreign cells here practically all have disappeared by the 22d day, leaving a mass of connective tissue. The absence of a round cell infiltration is the most marked difference in the process in the embryo as compared with that in the adult chicken. The process about the grafts in newly hatched chicks is characterized by a more active response of the connective tissue than in the adult and a more pronounced round cell infiltration than in the embryo.

EXPLANATION OF PLATES.

Plate 33.

Fig. 1. A graft of the Jensen rat sarcoma removed after 5 days in an adult chicken. A = the necrotic remains of the rat tissue surrounded by phagocytes; B = small mononuclear cells densely infiltrating the tissues around the graft.

Fig. 2. The edge of a rat sarcoma growing in an 18 day chick embryo, showing the absence of a defensive reaction on the part of the host's tissue.

Plate 34.

Fig. 3. The remains of a graft of rat sarcoma inoculated into a chick embryo on the 15th day and removed on the 20th day of incubation. A = the sarcoma cells showing many degenerate forms; B = the dense capsule of connective tissue surrounding and replacing the graft.

Fig. 4. A graft of rat sarcoma inoculated into an embryo on the 18th day of incubation and removed on the 21st day. A = a few surviving rat cells lying in a mass of necrotic material; the nuclei of the cells take the basic stain deeply; B = capsule of young connective tissue entirely walling off and replacing the graft.

FIG. 1.

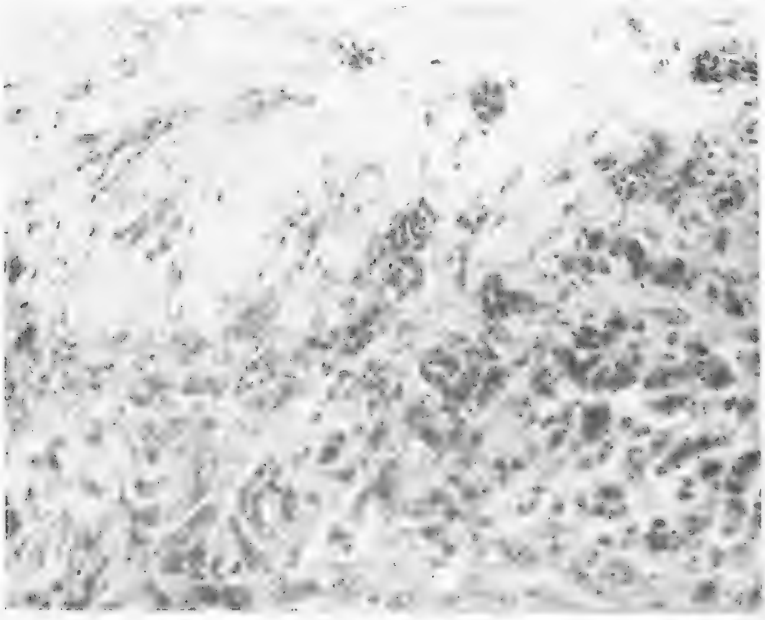


FIG. 2.

(Murphy: Studies in Tissue Specificity.)

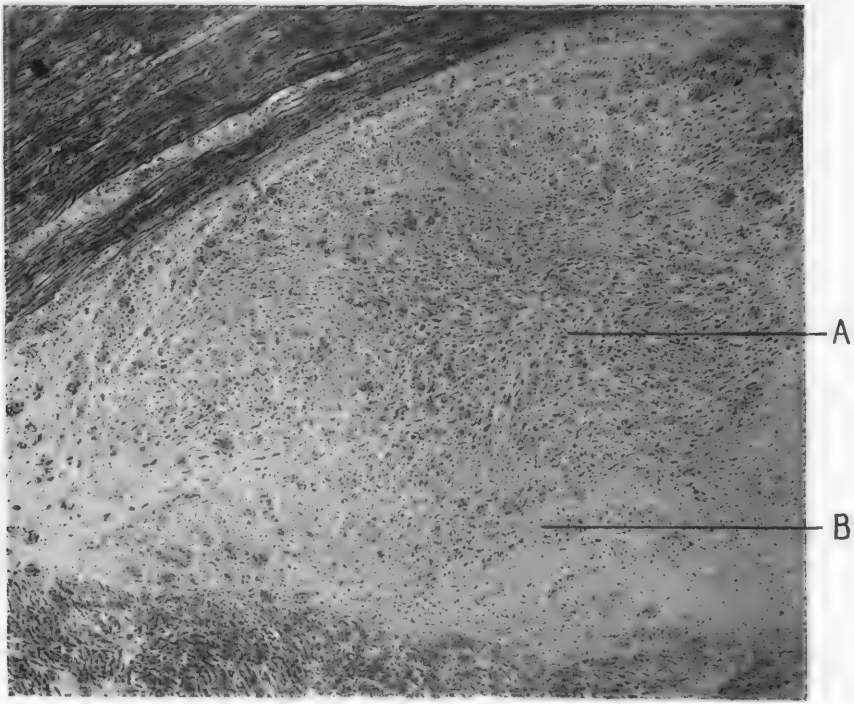


FIG. 3.

FIG. 4.

(Murphy: Studies in Tissue Specificity.)

THE FUNCTION OF THE SPLEEN IN THE EXPERIMENTAL INFECTION OF ALBINO MICE WITH BACILLUS TUBERCULOSIS.*

By PAUL A. LEWIS, M.D., and ARTHUR GEORGES MARGOT.

(*From the Henry Phipps Institute of the University of Pennsylvania, Philadelphia.*)

Experiments were begun in this laboratory three years ago to test the susceptibility of white rats and white mice to infection with *Bacillus tuberculosis*. It was expected that the animals would show a high degree of resistance to the infection, but it was hoped that they might react with such regularity as to make them available for testing various therapeutic measures. This expectation was in no way fulfilled. It developed that the animals were, on the whole, susceptible to infection, but that there was much irregularity in the reaction of individual animals.

The first experiments were made with rats. It was noted that a constant lesion in these animals was a great increase in the size of the spleen, which occurred whenever the animals survived two weeks or more. It was also noted that in several instances where the animals died in the second week after inoculation the spleen was small and extremely hemorrhagic. It seemed not impossible from a consideration of these facts that the spleen might be an important factor in the resistance of the animal to the infection, and that irregularity in the reaction of this organ might account in considerable degree for the irregularity of reaction of the animal as a whole. The same general facts were observed in a repetition of the experiments in mice.

A number of mice were splenectomized and later tested for their resistance, in comparison with intact controls. Contrary to expectation the splenectomized animals were found to have more than

* Read before the Pathological Section of the American Association for the Study and Prevention of Tuberculosis, Washington, May, 1913. Received for publication, December 6, 1913.

normal resistance. In fact a number of animals without spleen have survived for months after inoculation with amounts quickly fatal to normal animals. In this paper we shall present the data on which the foregoing statements are based, with such observations as we have so far made that bear upon the cause of the increased resistance.

We are aware of no literature bearing on the main points at issue although the virulence of the tubercle bacillus for rats and mice, and the pathological anatomy of experimental tuberculosis in these animals, has been the subject of considerable study.[1]

The removal of the spleen was carried out under ether anesthesia. After etherization the mice were tied out on a frog board. The abdominal region was shaved and the spleen delivered through a suitable incision. The main blood vessels were ligated in the omentum, and the organ was then cut off. In closing the laparotomy wound the muscles and skin were separately sutured with fine silk. The animals were kept for a number of hours after operation in a warm place, free from draught. The operation was well borne; a small proportion of the animals died, either under ether or, within a few days, from hemorrhage or peritonitis. Animals surviving beyond the first week seemed to be in good health. The inoculations have never been made in less than two weeks after operation, and have usually been done in the third week.

The cultures used for inoculation were of bovine type. Culture bovine C was isolated in 1912 from a case of spontaneous bovine tuberculosis. This culture is somewhat more virulent for mice than culture R_3, also of bovine type, isolated in 1911 from a human case of cervical adenitis.

The observations are presented in the following tables.

Table I illustrates the constancy and also the degree of enlargement of the spleen when rats are infected with fatal doses of tubercle bacilli.

Table II gives the results of all the inoculations made into normal mice with one, two, and five milligrams of culture bovine C. The culture has never failed to kill in these doses. The maxi-

[1] The literature is reviewed by Goldmann, E., Neue Untersuchungen über die äussere und innere Sekretion, Tübingen, 1912, 52.

TABLE I.

Relation of Spleen Weight to Body Weight in Rats.

Normal rats.		Tuberculous rats.[2]	
Body weight.	Spleen weight.	Body weight.	Spleen weight.
123 gm.	0.26 gm.	140 gm.	1.46 gm.
135 gm.	0.43 gm.	150 gm.	1.15 gm.
130 gm.	0.37 gm.	115 gm.	1.30 gm.
129 gm.	0.71 gm.	135 gm.	2.08 gm.
150 gm.	0.42 gm.	110 gm.	2.01 gm.
125 gm.	0.51 gm.	130 gm.	2.05 gm.
		115 gm.	1.60 gm.
		120 gm.	2.20 gm.
		125 gm.	1.01 gm.
		145 gm.	0.63 gm.
Average 132 gm.	0.45 gm.	128.5 gm.	1.57 gm.
Second Series. Average of Six Rats.			
70.6 gm.	0.20 gm.	Average weight lost.....	8.3 gm.

TABLE II.

Normal Mice. Culture Bovine C.

Dose.	Lived.	Exudate.	Spleen.	Liver.	Heart blood.	Lung.	Kidney.
1 mg.	14 dys.	+	+	−	−	−	−
1 mg.	5 dys.	+	+	−	−	−	−
1 mg.	13 dys.	+	+	+	o	+	+
1 mg.	13 dys.	+	+	+	+	+	+
1 mg.	13 dys.	+	+	+	o	+	+
1 mg.	14 dys.	++	+	+	+	+	+
1 mg.	14 dys.	++	+	+	+	+	+
1 mg.	19 dys.	++	+	+	o	+	+
1 mg.	19 dys.	++	+	+	o	+	+
1 mg.	19 dys.	++	++	+	+	+	+
2 mg.	10 dys.	+	+	+	o	o	o
2 mg.	13 dys.	+	+	+	o	+	o
2 mg.	14 dys.	+	+	+	o	+	+
2 mg.	20 dys.	+	++	++	+	+	+
2 mg.	20 dys.	++	++	+	+	++	+
2 mg.	33* dys.	++	+++	+	+	++	+
5 mg.	13 dys.	++	+	+	o	+	+
5 mg.	13 dys.	+	+	+	+	+	+
5 mg.	14 dys.	++	+	+	o	+	+
5 mg.	14 dys.	++	+	+	+	+	+
5 mg.	14 dys.	++	+	+	+	+	+

++ = many tubercle bacilli; + = few tubercle bacilli; o = no tubercle bacilli; — = examination for tubercle bacilli not made; * = nodules in lungs and heart muscle.

[2] The tuberculous animals were killed twelve days after the intraperitoneal inoculation of 10 mg. of the culture.

mum length of life was thirty-three days. At autopsy the animals frequently showed a small amount of thick yellow exudate in the omentum. The spleen was greatly enlarged. The one mouse that lived over thirty days showed numerous miliary nodules in the lungs. The nodules were softer and showed less organization than tuberculous masses of the same relative size as they are found in other species of experimental animals. The organs and frequently even the heart blood contained enormous numbers of tubercle bacilli.

TABLE III.

Splenectomized Mice. Culture Bovine C.

Mouse No.	Dose.	Lived.	Peritoneal dry exudate.	Peritoneal fluid.	Liver.	Kidney.	Lung.	Heart blood.	Remarks.
1	1 mg.	5 dys.	o	+	o	o	o	o	
2	1 mg.	18 dys.	o	o	o	o	o	o	
3	1 mg.	32 dys.	o	o	o	o	o	o	
4	1 mg.	34 dys.	+	−	+	o	−		
5	1 mg.	37 dys.	o	o	o	o	o	o	
6	1 mg.								Still living.
7	1 mg.								Still living.
8	1 mg.								Still living.
9	5 mg.	2 dys.	o	+	o	o	o	o	
10	5 mg.	29 dys.	+	−	+	o	+	+	Nodules in lungs and heart.
11	5 mg.	32 dys.	+	−	+	o	+	o	Nodules in lungs.
12	5 mg.	32 dys.	+	−	+	+	+	o	Nodules in lungs and liver.
13	5 mg.								Still living.

+ = few tubercle bacilli; o = no tubercle bacilli.

Table III gives the results of the first series of inoculations of splenectomized mice. It will be noted that two of the mice died within the first few days after inoculation. It is unlikely that the tubercle bacilli inoculated were responsible for the deaths. However, even if we include these presumably accidental losses, it is seen that the length of life in the splenectomized mice is much longer on the average than in the case of the normal mice, as shown in table II. Moreover, at the present writing (October, 1913), more than six months after inoculation, four of the animals are still alive and seem to be in perfect health. The increase of resistance, as brought out in this series, has been repeatedly demon-

strated in similar experiments or in slight modifications of them. It is of interest that these splenectomized mice, dying after the thirtieth day, show the same formation of nodules in the lungs that is found in the occasional normal mouse, surviving for an equal time. The bearing of this fact will be considered later.

It will further be noted that, as shown in tables II and III, the presence and distribution of the tubercle bacillus in the splenectomized animals that died was much more limited than in the intact animals. This suggested the possibility that in the splenectomized animals there might be a rapid destruction of tubercle bacilli which did not prevail in the intact animal. With this idea in mind we have studied the distribution of the tubercle bacillus in the first hours after intraperitoneal inoculation in splenectomized as compared with normal animals. The result of this study has been to show that in the normal animal, tubercle bacilli appear in the substance of the spleen, in the portal vein, in the liver, in the bile, and in the lumen of the small intestine within four hours after inoculation in sufficient numbers to be found readily. In splenectomized mice, on the contrary, we have never observed them outside the peritoneal cavity in less than seven days.

Within the peritoneal cavity there is no evidence of rapid lysis or increased phagocytosis. The splenectomized mice show more fluid exudate than the controls, but the significance of this fact is by no means apparent. It would seem, therefore, that the removal of the spleen has interrupted a path by which the tubercle bacillus is excreted from the normal mouse with some rapidity. These observations tend to emphasize the increased resistance brought about by the splenectomy, but in no way explain the cause of it.

In searching for an explanation of the increase of resistance, several possibilities demand consideration. In the first place it might be considered that the spleen formed a most favorable focus of infection as compared to the other organs of the body, and that with the removal of the organ much of the available food stuff is removed. Direct evidence as to the value to be assigned to the organ on the basis of these considerations is difficult to obtain.

The facts presented in regard to the limitation of the distribution of the bacilli in the splenectomized mice suggest that perhaps the

chief effect of the operation is to convert the general infection into a local one by interrupting mechanically a path of transmission. In order to test this supposition we have infected comparative series of animals in the pleural cavities. The results of this experiment as presented in table IV show that the increased resistance also prevails under these conditions. The infection tends to remain localized in the pleural cavities in this case.

TABLE IV.

Controls. Culture R_3. Intrapleural Inoculation.

Mouse No.	Dose.	Lived.	Pleural fluid.	Peritoneal exudate.	Spleen.	Liver.	Kidney.	Heart.	Lung.	Remarks.
1	1 mg.	24 dys.	+	+	+	+	+	0	++	Killed. Nodules in lungs.
2	1 mg.	27 dys.	++	+	+	+	+	0	++	Killed.
3	1 mg.	36 dys.	++	++	+	+	+	+	+	Pleural adhesions. Nodules in lungs.
4	1 mg.	37 dys.	++	++	+	+	+	+	+	Nodules in diaphragm. Adhesions of pleura.
5	1 mg.	39 dys.	+++	+	++	++	++	+	++	Nodules in mediastinum.
6	1 mg.	49 dys.	++	+	++	++	++	0	++	
7	1 mg.	49 dys.	+	+	++	++	++	0	++	Nodules in pleura.
8	1 mg.	49 dys.	++	+	++	+	++	+	+	Nodules in diaphragm.
9	1 mg.	61 dys.	++	+	++	+	+	+	++	Nodules in lung and heart.
10	1 mg.	61 dys.	++	+	++	+	+	0	++	Nodules in right axillary region surrounding a blood vessel.

Splenectomized Mice. Culture R_3. Intrapleural Inoculation.

11	1 mg.	25 dys.	++	0		0	0	0	+	Killed.
12	1 mg.	27 dys.	+	+		0	0	0	+	
13	1 mg.	48 dys.	++	0		0	0	0	+	Killed. Few nodules in lung.
14	1 mg.	70 dys.	+	0		0	0	0	++	
15	1 mg.	70 dys.	++	0		0	0	0	++	Few nodules in lung.
16	1 mg.	125 dys.	++	0		0	+	+	++	Many nodules in lung.
17	1 mg.	125 dys.	+	0		0	0	0	++	
18	1 mg.	133 dys.	++	+		0	0	0	+	Nodules in lung.
19	1 mg.	Living								
20	1 mg.	Living								

We are, therefore, forced to the supposition that following the removal of the spleen there is a general physiological effect which

increases resistance. It has been mentioned in commenting on the results presented in table III that the mice that die after several weeks in the splenectomized series show the formation of gross nodular lesions comparable to those seen in the occasional normal mouse which survives for an unusual period. It is also notable that in the peritoneal and pleural cavities there is a plastic exudate similar to that found in the intact animals. This plastic exudate is accompanied by more fluid in the case of the splenectomized animal, whether the infection is intrapleural or intraperitoneal, but this does not furnish an obvious clue to the cause of the changed resistance.

The character of the various exudates found in the splenectomized series of animals seems to indicate that the removal of the organ does not radically change what we may call the capacity for exudation possessed by the body. The infection in the animals without spleen tends to remain localized. Experiments in progress may, we hope, show on what this limitation of distribution depends.

One control experiment should be mentioned. It is a well known fact in clinical medicine that tuberculous peritonitis is often greatly benefited and sometimes apparently cured by laparotomy without continuous drainage. As a comparable operation we have therefore removed one kidney from a number of mice by transperitoneal operation. When subsequently infected with tubercle bacilli the nephrectomized mice have reacted as do normal animals.

The change of reaction that is produced in mice by the removal of the spleen is one of the few striking instances of an increased resistance to an experimental infection with *Bacillus tuberculosis*. It would be of great importance if it should be proven that the resistance of other species of animals could be similarly altered. Experiments on guinea pigs and dogs to test this point have so far failed to bring out any change in resistance.

SUMMARY.

Infection of rats and mice with *Bacillus tuberculosis* (bovine type) develops a splenic tumor as a typical lesion.

Removal of the spleen from mice (albino) greatly increases their resistance to the infection. This increased resistance cannot

be explained at present. The infection in the splenectomized mice tends to remain localized as contrasted with an almost septicemic type of disease which is usual in the normal animal. The animals of each group that live more than thirty days are apt to present typical exudative lesions. The removal of the spleen does not therefore grossly change what may be called the capacity of the body for exudation.

A CONTRIBUTION TO THE EPIDEMIOLOGY OF POLIOMYELITIS.*

By SIMON FLEXNER, M.D., PAUL F. CLARK, Ph.D., and HAROLD L. AMOSS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

As compared with the period prior to 1909, when the first experimental transmission of epidemic poliomyelitis to the lower animals was accomplished, our present knowledge of the pathology of the disease may be said to be comprehensive.[1] We now possess information in many ways accurate and full regarding the causative microörganism, its portal of entry into and paths of exit from the body, the period of its persistence in the tissues, the places of its location among the organs, the manner in which its presence brings about the characteristic lesions and symptoms of the infection, and certain important immunity reactions which it displays. The data upon which this knowledge is based is being extended by experiment; but up to the present the experimental studies have not yielded results that illuminate particularly the epidemiology of the affection. The observations recorded in this paper are believed to bear upon that aspect of the disease.

PATHOGENICITY OF THE VIRUS.

Hitherto by the virus of poliomyelitis there has been understoód an emulsion or filtrate prepared from infected tissues, originally of human origin, capable of transmitting poliomyelitis to monkeys. As long as the virus had not been cultivated artificially or rendered visible no other criteria than successful inoculation served to identify it. In spite of the cultivation of the organism outside the body the above definition holds in practice, for the reason that cul-

* Received for publication, December 15, 1913.

[1] Landsteiner, K., and Popper, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1909, ii, 377. Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 1639.

tivation is still difficult and in inexperienced hands uncertain, and because also less account can be taken of the artificially cultivated virus in considering pathogenicity as its virulence is readily lost.[2]

Somewhat conflicting results have been recorded as to the power of infected human tissues (usually parts of the central nervous organs) to convey paralysis to monkeys. The number of successful implantations has varied from one half or less of the specimens tested[3] to all those inoculated.[4] No conclusion regarding the virulence of the original materials can be drawn from these figures which often are based upon a few tests in which wide differences in method play a part. One exception only to this generalization may have to be made. Of all countries visited recently by epidemic poliomyelitis Sweden seems to have suffered most severely. Elsewhere than in Sweden it has been found difficult to communicate experimental poliomyelitis to monkeys with filtered washings of the nasopharyngeal mucous membranes from acute cases.[5] The deduction seems obvious, therefore, that the Swedish virus, as it exists in nature, is more active for monkeys than the strains occurring in other countries. And yet the conclusion cannot be accepted unreservedly since even small differences in the methods employed for preparing the washings may account for the discrepancy. Thus far in Sweden the virus, which is believed to be carried by healthy persons, has not been transmitted to monkeys so as to set up the typical experimental paralysis, in spite of many trials, while it has been inoculated successfully in the United States.[6] There is nothing inherently improbable in the supposition that the virus of immediate human origin in one place should be more active because better adapted for monkeys than elsewhere at the same period. But in becoming adapted to the monkey the virus undergoes profound modifications; therefore caution is needed before the view is

[2] Flexner, S., and Noguchi, H., *Jour. Exper. Med.*, 1913, xviii, 461.

[3] Landsteiner, K., in Kolle-Wassermann, Handbuch der pathogenen Mikroorganismen, 2d edition, Jena, 1913, viii, 427.

[4] Flexner, S., and Clark, P. F., *Jour. Am. Med. Assn.*, 1911, lvii, 1685.

[5] Kling, C., Wernstedt, W., and Petterson, A., *Ztschr. f. Immunitätsforsch., Orig.*, 1911–12, xii, 316, 657; 1912, xiv, 303.

[6] Flexner, S., Clark, P. F., and Fraser, F. R., *Jour. Am. Med. Assn.*, 1913, lx, 201.

adopted that such already modified strains exist in nature. And yet it appears that in Sweden and probably in still other countries the virus fluctuates considerably in its effects upon human beings, as indicated by the varying prevalence, severity, and mortality of the epidemics of poliomyelitis.

By successive passages of human strains of the virus through monkeys a high degree of virulence may be attained for this species. How great the changes are that take place can be inferred only, since in the inoculation of filtered extracts of the nervous and other organs we cannot actually measure the number of micro-organisms introduced. But where the original materials may prove infective only when in a state of an emulsion of which several cubic centimeters may be required, ultimately 0.1 to 0.001 of a cubic centimeter of Berkefeld filtrate may suffice to cause paralysis. The rise in virulence thus indicated is not determined merely by the smaller effective dose but also by the circumstance that while at the beginning of the adaptation the proportion of monkeys developing paralysis is smaller and the number of recoveries after paralysis larger than at a later period, once the adaptation has been accomplished all the animals inoculated tend to become paralyzed and to succumb to the disease.[7]

After it had acquired a state of high virulence the virus retained maximal activity for monkeys over a long period of time. But whether this maximum becomes a fixed quality was not known. This point is one of fundamental importance as regards, among other things, the question of the causes responsible for the rise and fall of epidemic waves of the disease in nature. We have carried a particular strain of the poliomyelitic virus (M A) through a series of monkeys beginning in the autumn of 1909[8] and ending in the autumn of 1913. At the outset the virus conformed in activity to the description given: at first infection was irregular and recovery after paralysis not uncommon. After several passages adaptation of the virus was secured and infection followed more regularly and then constantly upon inoculation for which small doses of the filtrate

[7] Flexner, S., Huxley Lecture, *Lancet*, 1912, ii, 1271, and *Science*, 1912, xxxvi, 685.

[8] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 1639.

sufficed. Recovery at first rarely and later never occurred.[9] For precautionary reasons the inoculations were usually made in duplicate or multiple series. At the same time portions of the spinal cord and brain from infected animals were regularly set aside in 50 per cent. glycerin in the refrigerator, so that if an inoculated monkey succumbed to an intercurrent disease,—dysentery, pneumonia, or tuberculosis,—as occasionally happens, the strain would not be lost.

The M A strain retained maximal virulence during a period of about three years, from the winter of 1909 to the winter of 1912, when a change became apparent. The power to infect began to fluctuate, while among the paralyzed animals recovery began to take place. Once begun the infective power diminished more and more although not regularly. Even at this time several successive inoculations might be succeeded by infection and death. But gradually and somewhat irregularly the virulence deteriorated more and more until the state of the virus resembled in its infecting power that of the original human material from which it was derived. At first the filtrates and only later the emulsions acted irregularly and unreliably. The infective power was not, however, completely abolished, for paralysis could still be induced in occasional animals by means of large doses of filtrates or emulsions, but by no means certainly, irrespective of the size of the dose administered.

During the long period of maximal infectivity the virus seemed to have acquired established or fixed virulence. It now became obvious that fixation of pathogenic effect in the sense in which this term is applied to the vaccine or rabic virus did not occur. Moreover, the return of the maximal virus to a state similar to that of the original human source is apparent only at the present time since thus far no tendency to rise again has been noted in the deteriorated strain. This is true of all the collateral specimens of the M A strain carried through the several series, the deterioration not being confined to a single specimen of the strain. Whether after a resting period a second enhancement can be accomplished by a new series of passages cannot of course be predicted. But

[9] Flexner, S., and Lewis, P. A., *Jour. Exper. Med.*, 1910, xii, 227.

that the deterioration began at a time considerably previous to its discovery is shown unmistakably by our attempts to secure a vigorous strain by returning to glycerinated specimens set aside during the still active period of the virus. After a varying number of passages fluctuation again set in and a similar irregularity in pathogenic effects appeared necessitating abandonment of the series. Long glycerination itself preserves the virus in the state of activity possessed at the time it was set aside. By resorting to a specimen (K) originally also adapted in 1909[10] and passed through an occasional monkey at long intervals, a highly virulent strain was again secured for experimental purposes. If a comparison of the two strains M A and K, adapted at about the same time, is permissible the conclusion to be drawn from their relative present states of activity is to the effect that frequent and long continued passage through monkeys finally brings about a depression of virulence, while preservation in a state of latency for a period equally great exerts no depressing action.

When the M A virus became highly potent the inoculation series were continued in two general ways. What was termed the regular series was designed merely to secure a constant passage virus. For this purpose Macacus monkeys were systematically inoculated, usually intracerebrally, with 1 or 2 c.c. of a Berkefeld filtrate prepared from a 5 per cent. suspension of the fresh medulla and spinal cord of a recently paralyzed animal. The disease thus caused ran the typical course of experimental poliomyelitis. The rule was to etherize the paralyzed animals within this series. Coincidentally with the regular series other series of inoculations were conducted in the course of the study of special aspects of the problem of poliomyelitis. The filtrate for this second group of experiments was usually given in smaller doses. The active virus caused paralysis in quantities of filtrate varying from 0.1 to 0.01 c.c. or less; hence the doses employed ranged from 0.1 to 0.5 c.c., depending on the purpose of the injection. The incubation period of the series receiving the smaller doses was somewhat greater than that of the regular series, but the final result was the same. In some instances intrasciatic[11] and combined intrasciatic and intraperitoneal injections[12] were employed. An active virus causes infection by intraneural injection almost as constantly as by intracerebral inoculation. Falling off in virulence is expressed in (a) failure to cause paralysis, (b) mild infection followed by recovery, and (c) by atypical symptoms and clinical course, followed by either recovery or delayed paralysis and death. The typical course is characteristic. Following an

[10] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 1913.

[11] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 1913.

[12] Leiner, C., and von Wiesner, R., *Wien. klin. Wchnschr.*, 1909, xxii, 1698; 1910, xxiii, 91.

incubation period of from five to seven days there succeed excitement, general tremor, weakness, and then paralysis of muscles affecting first the extremities, next the trunk, and lastly the respiration. Exceptionally the muscles of respiration are affected early and convulsions and death occur before paralysis of the extremities is noted. When the course is atypical either reinoculation or microscopical examination of the spinal cord may be required to establish the infection.

The fact of the loss of power of the M A strain of the virus is brought out in table I. Other instances showing this loss have not been included. These were examples of border-line inoculation with smaller doses of virus; but they serve to exclude still further any effect of resistance as such as being a determining factor among the monkeys.

TABLE I.

Illustrative Examples of Weakened Virus.

Date of inoculation.	Materials inoculated.	Clinical result.	Remarks.
Aug. 24, 1912	Emulsion of spinal cord	Typical paralysis	Death on 6th day.
Aug. 26, 1912	Filtrate of preceding emulsion	No effect	
Sept. 1, 1912	Emulsion	Atypical paralysis	Death on 8th day.
Sept. 23, 1912	Filtrate	Atypical paralysis	Recovery; immune.
Oct. 7, 1912	Emulsion	Atypical paralysis	Death on 11th day.
Dec. 9, 1912	Filtrate intrasciatic	No effect	Two animals used.
Dec. 18, 1912	Filtrate	Atypical paralysis	Recovered.
Jan. 21, 1913	Emulsion intrasciatic	No effect	Two animals used.
Feb. 8, 1913	Filtrate 0.1, 0.2, 0.3 c.c. intracerebral	Paralysis not strictly typical	Three animals used.
Apr. 17, 1913	Filtrate	Typical paralysis or no effect	Of 4 animals 2 escaped paralysis.
July 2, 1913	Emulsion	Slight ataxia; no progress	Recovery.
July 9, 1913	Emulsion	Atypical paralysis	Recovery.
July 10, 1913	Emulsion	Partial paralysis	Recovery with residual paralysis.
July 19, 1913	Filtrate	Typical paralysis; prostrate	Recovery with residual paralysis.
July 21, 1913	Filtrate	Typical paralysis; prostrate	Recovery with residual paralysis.
Aug. 26, 1913	Filtrate	Ataxic; partial paralysis	Recovery with residual paralysis.
Sept. 9, 1913	Emulsion	No paralysis	Four other animals inoculated with filtrate from this emulsion without effect.
Sept. 10, 1913	Filtrate	Partial paralysis; rapid partial recovery	Recovery with residual paralysis.
Oct. 23, 1913	Emulsion	Partial paralysis	Recovery with residual paralysis.

BEARING ON EPIDEMIOLOGY.

We do not possess a generally acceptable theory to account for the epidemic waves of disease. What is required is an adequate explanation of the initial rise, persistence, and the final fall of the wave as represented by the varying number of the affected. That mere presence of the microbic causes of disease does not suffice to produce epidemics has long been known. It is just the discrepancy between the occurrence of the microbic causes in sporadic cases of potentially epidemic diseases and the absence of true epidemics that has led to the formulation of the hypothesis of concomitant causes of von Pettenkofer and of Nägeli. While the one supposes a necessary ripening of the microbic agent in the earth as a prerequisite, the other invokes the coöperation of a second although unknown but subsidiary microörganism.[13] The subject has not been rendered essentially more comprehensible by the discovery of the healthy and chronic carriers of infectious microörganisms, or by the more ready detection of so called abortive cases of infection. Indeed, these discoveries only add to the perplexity since they prove that potentially infective microörganisms capable of starting epidemics are more frequently present in our surroundings than has hitherto been supposed.

Perhaps a factor which has not up to the present been sufficiently considered is that of variations among the microörganisms themselves that may be directly responsible for the production of epidemics. That microörganisms, along with all living things, tend to vary in their biological properties, has long been known, but it is only recently that these variations have been recognized as constituting mutations.[14] The variations thus far studied relate chiefly to (a) colony formation and fermentation effects, and (b) serum and drug reactions of fastness, while variations affecting the quality of virulence have been little considered in this respect.

The number of examples known in which mutation in the quality of virulence has occurred is already considerable. Thus per-

[13] Gotschlich, E., in Rubner, M., von Gruber, M., and Ficker, M., Handbuch der Hygiene, Leipzig, 1913, iii, pt. 1, 206.

[14] Müller, R., *Ztschr. f. indukt. Abstammungs- u. Vererbungslehre,* 1912, viii, 305.

manent alterations of this character have been produced in vaccine virus and the fixed rabic virus. Not a few pathogenic bacteria may be changed profoundly in virulence by animal passages, oftenest with the effect of intensification but not infrequently with the contrary effect. Even in artificial cultures enhancement as well as diminution of virulence has been noted and particularly in the case of plague bacilli and meningococci.[15] The modifications in virulence appear at one time quickly or even suddenly and at another develop gradually. There is no doubt that under many natural conditions the passage of infectious microörganisms rapidly from animal to animal or person to person leads to great enhancement of virulence as, to mention one example only, in the pneumonic form of the plague. There exists experimental foundation for the belief that during the rise the microbic causes are more virulent than during the fall of epidemics. The questions to be answered are whether it is this variation, perhaps mutation, among the potential microbic causes of epidemics that is responsible for the waves mentioned, and if so what the nature of the agency is that brings about the mutational changes. It is because the data bearing on the cycle of pathogenicity of the M A virus seem to offer answers to these important questions that they have been set down here in detail.

At the outset the virus of human poliomyelitis possesses relatively weak pathogenic action for monkeys. By means of a few passages the infective power rises and soon a maximum is reached which endures for several years. Ultimately, the infective power falls off and soon becomes greatly diminished, so that finally the power is no greater than at the outset. This succession of phenomena dependent on changes in virulence finds a counterpart in the phenomena noted during the rise, persistence, and then fall of numbers of cases, among man and animals, that constitute epidemics of disease. Moreover, the fluctuations in virulence upon which the phenomena in the first instance depend are the product, as far as can now be determined, of causes acting upon the M A virus from within, that is, the result is due to internal rather than to external effects. In the

[15] Gotschlich, E., in Kolle-Wassermann, Handbuch der pathogenen Mikroorganismen, 2d edition, Jena, 1911, i, 166, 167.

course of the long propagation of the M A virus through monkeys the species was constant and the methods employed for inoculation remained uniform. These causes, whatever their nature, operate to produce a cycle of activity indicated by rise, fixation and fall in infecting power. And this is the cycle, apparently, that many epidemics pass through in the course of their appearance and disappearance.

There exists another fact inherent in all epidemics which lies, however, outside the present consideration, namely, the varying number of susceptible persons who fall victims to the prevailing disease. Our observations bear upon the conditions which make epidemics possible rather than those which determine their actual extent.

In the light of this presentation the part played by sporadic and abortive cases and of the microbe carriers of potentially epidemic diseases becomes more comprehensible. We may consider this class of infected persons or animals as carrying specific microörganisms lacking high virulence for their respective kind. And we may begin to see how the conversion, through favoring causes, of microörganisms of low into others of high virulence, may be the signal for the appearance of epidemics, not necessarily confined to one place but, possibly, arising almost simultaneously in separated and even remote places when the conditions are similar; just as, on the other hand, the immediate transportation of already elevated microörganisms from a place in which an epidemic is already prevailing to new places may start similar severe outbreaks there.

SUMMARY.

A strain of the poliomyelitic virus was propagated in monkeys for four years, during which time it displayed three distinct phases of virulence. The several phases covered different periods of time. At the outset the virulence was low, but by animal passages it quickly rose to a maximum; this maximum was maintained for about three years, when, without known changes in the external conditions, a diminution set in and increased until at the expiration of a few months the degree of virulence about equalled that present

at the beginning of the passages in monkeys. The cycle of changes in virulence is correlated with the wave-like fluctuation in epidemics of disease which also consist of a rise, temporary maximum, and fall in the number of cases prevailing. And an explanation of epidemics of disease is inferred in variations or mutations among the microörganismal causes of disease affecting chiefly the quality of their virulence.

A CONTRIBUTION TO THE PATHOLOGY OF EPIDEMIC POLIOMYELITIS.*

By SIMON FLEXNER, M.D., PAUL F. CLARK, Ph.D., and HAROLD L. AMOSS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

In the course of our studies on experimental poliomyelitis we have collected certain data on the pathology of the disease which seem of sufficient interest to warrant publication. They relate to the distribution of the virus in the nervous tissues outside the cerebrospinal axis and its bearing on the pathogenesis of poliomyelitis, the resistance of the virus to glycerin, phenol, and freezing, and to immune bodies in the cerebrospinal fluid after recovery from infection.

THE VIRUS IN THE SPINAL, GASSERIAN, AND ABDOMINAL SYMPATHETIC GANGLIA.

That the virus of poliomyelitis is not present exclusively in the affected regions of the spinal cord but occurs also constantly in the brain was observed early in the course of the experimental inoculations of monkeys.[1] Later the virus was found to be present in the intervertebral ganglia of infected monkeys.[2] But no systematic study has been made from this point of view of the nervous organs that lie outside of and at a distance from the cerebrospinal axis. This we have now done with the results to be described, which have a bearing on the prevailing views on the pathogenesis of poliomyelitis.

The inoculation tests prove that the virus exists in the intervertebral, Gasserian, and abdominal sympathetic ganglia. Illustrative protocols follow of several positive inoculation experiments.

* Received for publication, December 18, 1913.

[1] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 1639.

[2] Flexner, S., and Clark, P. F., *Jour. Am. Med. Assn.*, 1911, lvii, 1685.

Protocol 1. Intervertebral Ganglia from the Monkey.—Feb. 2, 1911. Several intervertebral ganglia were removed aseptically from a monkey that had been inoculated intracerebrally with M A virus and become paralyzed. The ganglia were emulsified in 2 c.c. of salt solution and injected intracerebrally into a *Macacus rhesus*. Feb. 7. Back muscles weak. Feb. 8. Extremities and back paralyzed. Feb. 9. Death. Microscopical examination of the spinal cord showed typical lesions of poliomyelitis.

Protocol 2. Intervertebral Ganglia from the Monkey.—Dec. 26, 1913. Several intervertebral ganglia were removed aseptically from a monkey that succumbed to inoculation of K virus. An emulsion in salt solution was injected into the sciatic nerve and peritoneal cavity. Jan. 1, A. M. Excitability; ataxia. P. M. Legs paralyzed. Jan. 2. Prostrate, died. Lesions of poliomyelitis.

Protocol 3. Intervertebral Ganglia from a Child.—Aug. 8, 1911. Several spinal ganglia which had been taken from a fatal case of acute poliomyelitis in a child and placed in glycerin a few days previously were emulsified in salt solution and injected intracerebrally into a *Macacus rhesus*. Aug. 19. Excitability; paralysis of right arm. Aug. 25. Paralysis of left leg. Sept. 1. Condition improving. Finally recovery occurred, with residual paralysis of one arm.

Protocol 4. Gasserian Ganglia from the Monkey.—Nov. 15, 1913. Four sets of Gasserian ganglia were removed from monkeys dead of K virus. They were emulsified in salt solution and injected into the right sciatic nerve and the peritoneal cavity of a *Macacus rhesus*. Dec. 2. Excitability; tremor; paralysis of right leg. Dec. 3. Both legs paralyzed; back weak; protects right arm. Dec. 4. Both arms weak. Dec. 5. Prostrate. Etherized. Lesions of poliomyelitis.

Protocol 5. Sympathetic Ganglia from the Monkey.—Nov. 15, 1913. Four sets of the abdominal sympathetic ganglia (solar plexus) removed from monkeys dead of K virus (same as preceding) were emulsified in salt solution and injected into the left sciatic nerve and the peritoneal cavity of a *Macacus rhesus*. Nov. 26. Excitability; weakness; paralysis of left leg. Nov. 27. Left arm weak; right arm weaker. Nov. 28. Left arm paralyzed; back weak. Nov. 29. Prostrate. Etherized. Lesions of poliomyelitis.

Protocol 6. Sympathetic Ganglia from the Monkey.—Dec. 3, 1913. Sympathetic ganglia were removed from a monkey succumbing to K virus and suspended in salt solution which was injected into the left sciatic nerve and peritoneal cavity. Dec. 9. Excitability. Left arm weak, and later in the day paralyzed. Dec. 11. Weakness of back. Dec. 12. Left leg paralyzed; right leg weak; right arm weak. Dec. 14. Prostrate. Etherized. Lesions of poliomyelitis.

The protocols show that the ganglia inoculated contain the active virus of poliomyelitis. In view of the frequency with which the demonstration can be made with the ganglia and the rarity with which it has been made with non-nervous tissues, with possibly the one exception of the mesenteric lymph nodes, we may conclude again that the virus is strongly neurotropic. Apparently the virus is not stored in the peripheral nerves[3] but seeks the parenchymatous nerv-

[3] Landsteiner, K., in Kolle-Wassermann, Handbuch der pathogenen Mikroorganismen, 2d edition, Jena, 1913, viii, 445.

ous organs in which to multiply. But the selection among the latter appears to be small, since the virus occurs regularly not only in the spinal cord but in the brain and the several ganglia mentioned, of which some are remote from the cerebrospinal axis.

Moreover, the localization of the virus in the ganglia takes place early. Monkeys in the preparalytic stage of infection, before any detectable weakness of muscles has set in but while in the state of excitement, already show lesions of the intervertebral ganglia. Thus far the other ganglia have not been studied in the early period of the infection.

The histological lesions in the ganglia correspond accurately with those in the spinal cord: they are partly interstitial and perivascular, partly parenchymatous. The former extend in part from the pial investment of the ganglia to the fibrillar supporting tissue and small blood vessels which may be the seat of diffuse or of nodular accumulations of mononuclear cells. The degree to which the nerve cells are destroyed is variable. At times they escape entirely even when the interstitial changes are marked; again they are widely degenerated, or even necrotic, and have become invaded with neurophages. So far as the changes in the spinal ganglia are concerned they appear to arise through an extension of the cellular invasion from the subarachnoid spaces of the spinal cord. The changes in the Gasserian are less severe than those of the spinal ganglia, and the mode of invasion is not so evident. The cellular accumulations within the abdominal ganglia (solar plexus) are least marked and so constantly perivascular that infection by way of the blood is indicated. The nerve cells exhibit the slightest lesions of all the ganglia studied.

In view of the constancy with which the sensory ganglia are the seat of early and even profound histological changes undoubtedly caused by the presence of the virus, it becomes questionable whether any considerable support still exists for the belief that poliomyelitis is essentially a disease of the anterior grey matter of the spinal cord.[4] It would appear rather to be a disease of the nervous system in its entirety, although considered clinically it receives its significance from the muscular paralysis that is caused. Nor can it be held that

[4] Landsteiner, K., *loc. cit.*, p. 439.

the virus of poliomyelitis possesses a special affinity for nerve cells as such, since the lesions which it causes are impressed almost indifferently upon nerve cells and non-nervous elements within the nervous organs. Indeed, accurate studies of the cerebrospinal fluid in preparalytic and abortive human cases of poliomyelitis emphasize the early involvement of the leptomeninges in the pathological process.[5]

The experiments in which intrasciatic injections were made indicate that the virus travels to the spinal cord by the lymphatic channels and brings about paralysis first on the side of injection and later on the opposite side.[6] It is interesting to find that the virus may ascend by means of the lymphatics of the cord to the cervical level and set up paralysis in an arm before the leg into which the virus was injected shows distinct evidences of paralysis (protocol 6).

RESISTANCE OF THE VIRUS TO GLYCERIN, PHENOL, AND FREEZING.

Glycerin.—It was early noted that the virus of poliomyelitis resists glycerin for a time.[7] Römer and Joseph[8] found specimens still active after five months' glycerination. We have tested a specimen of virus K and found that it retained its activity undiminished after twenty-five months in 50 per cent. glycerin, the preparation having been kept constantly in the refrigerator at about 4° C.

Protocol.—Sept. 24, 1913. A *Macacus rhesus* was inoculated intracerebrally with an emulsion of spinal cord containing virus K, which was derived from a paralyzed monkey on Aug. 21, 1911. On Oct. 1, or seven days after the inoculation, the animal was excitable, and the arms and back were weak. Oct. 2. Paralysis of extremities and back complete. Etherized. Lesions of experimental poliomyelitis.

The M A strain withstands glycerination probably as well as the K strain. A Macacus inoculated with an emulsion prepared from nervous tissue in glycerin for eight and eleven months respectively developed paralysis in characteristic manner.

[5] Draper, G., and Peabody, F. W., *Am. Jour. Dis. Child.*, 1912, iii, 153.

[6] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 1913.

[7] Flexner, S., and Lewis, P. A., *Jour. Exper. Med.*, 1910, xii, 227.

[8] Landsteiner, K., and Levaditi, C., *Compt. rend. Acad. d. sc.*, 1910, cl, 131; *Ann. de l'Inst. Pasteur*, 1910, xxiv, 833. Römer, P. H., and Joseph, K., *München. med. Wchnschr.*, 1910, lvii, 1059.

Phenol.—That the poliomyelitic virus withstands phenolization was shown by Kraus,[9] who proposed first to treat paper filtrates of the infected spinal cord of monkeys with 0.5 per cent. and later with 1 to 1.5 per cent. solutions of phenol in order to diminish their potency and thus to make possible their employment for purposes of active immunization. Since the inoculations of the phenolized virus were made exclusively into subcutaneous tissues no index of the degree of its infectiousness was obtained. Macacus monkeys react irregularly to subcutaneous injections of the virus even when highly active. Flexner and Clark[10] employed phenol for the purpose of rendering bacteria-free the virus contained in tonsillar and adenoidal tissues, and to prepare them for inoculation into monkeys. They observed no injurious action of 0.5 per cent. phenol in the virus at the expiration of fourteen days' exposure. The long survival of the virus in undiminished activity in glycerin suggested the testing for activity of a specimen of human spinal cord and brain put aside in the refrigerator fifteen months before in 0.5 per cent. phenol.

Protocol.—Oct. 20, 1913. A *Macacus rhesus* was inoculated into the sciatic nerve and peritoneal cavity with an emulsion obtained from the central nervous tissues from an actively fatal case of poliomyelitis in a child, and preserved since July 25, 1912, in 0.5 per cent. phenol. Nov. 5. Paralysis of legs and weakness of back; arms strong. Nov. 6. Prostrate. Etherized. Lesions of poliomyelitis present.

Two experiments were made to determine whether 0.5 per cent. phenol acting upon the virus contained within a Berkefeld filtrate almost devoid of coagulable protein would affect the activity of the virus. The mixtures were allowed to remain at 22° C. for twenty hours and five days respectively, at the expiration of which period both were found to be highly infectious and capable of causing experimental poliomyelitis after average incubation time.

Freezing.—In contrast to the long survival of the virus in an active state in glycerin and phenol is its shorter survival when the tissues containing it are kept continually frozen at a temperature of —2° to —4° C. Under these conditions we have found the virus

[9] Kraus, R., *Wien. klin. Wchnschr.*, 1910, xxiii, 233; *Ztschr. f. Immunitätsforsch.*, 1911, ix, 117.

[10] Flexner, S., and Clark, P. F., *Jour. Am. Med. Assn.*, 1911, lvii, 1685.

active at the end of six weeks; but infection did not occur when material frozen for one and a half to three years was employed for inoculation.

Neutralization Tests with Cerebrospinal Fluid.—After recovering from experimental poliomyelitis monkeys are not subject to reinoculation; an active immunity has developed.[11] This state of immunity is associated with the occurrence in the blood of principles that neutralize the virus *in vitro*.[12] Since the poliomyelitic virus produces profound alterations in the cerebrospinal fluid and this fluid gains ready access to the interstices of the central nervous tissues, tests were carried out to determine whether neutralizing antibodies existed within it. It is known that antibodies are not secreted in appreciable quantities into the cerebrospinal fluid; hence any considerable quantity that might be present there would probably have been produced locally.[13] The fluids selected for the tests were obtained from patients who had recently suffered from typical paralytic poliomyelitis from which they were convalescent, and were scrupulously free from blood. The acute symptoms had begun from one to three months prior to the collection of the cerebrospinal fluid.

Seven fluids were tested. The experiments were practically uniform. 0.1 of a cubic centimeter of highly active filtrate was mixed with from two to three cubic centimeters of the cerebrospinal fluid, the mixtures were incubated for two hours at 37° C., and placed in the refrigerator over night. The inoculations were intracerebral. Six of the monkeys developed typical paralysis after the average incubation period. One monkey remained perfectly well, from which it may be concluded that neutralization of the virus was accomplished. The child from whom the neutralizing specimen was obtained had been attacked about six weeks before. It would therefore appear possible, but highly unusual, for neutralizing immunity principles to be present in the cerebrospinal fluid during convalescence from epidemic poliomyelitis.

[11] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1910, liv, 45.
[12] Levaditi, C., and Landsteiner, K., *Compt. rend. Soc. de biol.*, 1910, lxviii, 311.
[13] Flexner, S., *Jour. Am. Med. Assn.*, 1913, lxi, 447.

SUMMARY.

The virus of poliomyelitis is neurotropic, and localizes, and probably is capable of multiplying in the extramedullary parenchymatous nervous organs. It has been demonstrated by inoculation tests in the intervertebral, Gasserian, and abdominal sympathetic ganglia.

All the ganglia show histological lesions, more or less severe, similar to those of the spinal cord and brain. The severest occur in the intervertebral ganglia, those next in severity in the Gasserian, while the mildest appear in the abdominal sympathetic ganglia. The interstitial lesions predominate over the parenchymatous, and in preparalytic stages the intervertebral ganglia show interstitial lesions, especially pronounced at the pial covering.

Epidemic poliomyelitis is a general disease of the nervous system, although the most prominent and important symptoms are those following injury to the motor neurones of the spinal cord and brain.

The virus of poliomyelitis is highly resistant to glycerin, in which it survives for more than two years; to 0.5 per cent. phenol, in which it survives for more than one year; while it succumbs after having been kept frozen constantly for several months.

It is unsafe to employ phenol to modify the virus of poliomyelitis for the purpose of active immunization.

The cerebrospinal fluid of convalescents tends to be devoid of the neutralizing immunity principles for the virus of poliomyelitis, although they may exceptionally be present within this fluid. Doubtless the immunity principles are not produced locally in the nervous tissues, but elsewhere in the body, and are carried to the nervous organs by the blood.

A NOTE ON THE ETIOLOGY OF EPIDEMIC POLIOMYELITIS.*

By HAROLD L. AMOSS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

PLATE 35.

Flexner and Noguchi[1] have cultivated a minute microörganism from the central nervous organs of human beings and monkeys that have succumbed to epidemic poliomyelitis, and Noguchi has perfected a method by which the parasite may be stained in film preparations and sections prepared from infected tissues. Either because of their minute size or because of the manner of distribution, the demonstration of the stained microörganisms in sections of the nervous tissues has been accomplished with difficulty. Moreover, one cannot be sure of the presence of a single organism in tissues where so many granules confuse the microscopic picture. I have therefore endeavored to arrive at more certain results by employing for the sections to be stained portions of nervous tissues which have previously been incubated for a sufficient time to allow the parasites to increase in number.

For the cultivation of the microörganism described by Flexner and Noguchi fragments of the brain are placed in sterile ascitic fluid together with a piece of sterile rabbit kidney, the whole being overlaid with paraffin oil. At the expiration of ten days the fluid culture is examined for growth. When uncontaminated with extraneous bacteria the medium shows almost no changes; and it may even remain unchanged when certain contaminating bacteria are present. The extraneous bacteria are detectable (a) by microscopical examination and (b) by subculture in the usual culture media. The smaller microörganism thus far cultivated only from poliomyelitic tissues is readily distinguished from ordinary bacteria.

* Received for publication, December 15, 1913.

[1] Flexner, S., and Noguchi, H., *Jour. Exper. Med.*, 1913, xviii, 461.

But many of the cultures set up as above stated remain permanently free from stainable microörganisms within the fluid. To obtain initial growth of the minute parasite requires much care and patience. The fragment of brain retains its form for many weeks and autolyzes slowly.

By this method of cultivation it is supposed that the minute microorganism first multiplies in the fragment of brain from which it gradually enters the ascitic fluid. It is probable therefore that growth may take place within the brain tissue and not in ascitic fluid, where for many reasons the conditions happen not to be favorable for multiplication. Since the parasite is subject to autolysis it may escape detection in microscopical preparations, because the small number that have entered from the brain tissue may have perished, or they may be too greatly diluted to be readily found.

However, it should be possible to detect the multiplied microörganism in the fragment of brain tissue itself. As a matter of fact this has been accomplished, and the minute parasites have been found in such states as to show conclusively that they have multiplied post mortem.

Flexner and Noguchi pointed out that the minute globoid bodies which they cultivated appeared within the nervous tissues in single and double form, but not in chains or distinct masses such as occur in artificial cultures. We may assume therefore that during postmortem multiplication within the tissues both the chain and mass form would arise, as has been actually observed. The procedure employed for the demonstration of the bodies in the incubated tissue follows.

Monkey A.—Inoculated intracerebrally on the left side with 0.3 c.c. of a Berkefeld filtrate of M A virus, began to show symptoms of poliomyelitis on the seventh day, and ran a typical course. The animal became prostrate on the ninth day and was etherized on the thirteenth. Small portions (about 1 by 1 by 2 cm.) of the right brain were placed in tubes of kidney-ascitic fluid culture medium and incubated at 37° C. Beginning with the second day and daily thereafter a tube was taken out and the fluid replaced by molten agar, and the mass containing the piece of brain was poured immediately into a small dish. When the agar had congealed small pieces of the brain were cut and dropped into pyridin-lucidol[2] for hardening, according to the method described by Szésci, and finally blocked

[2] Lucidol is the name given by Szésci to benzoyl peroxide. Szésci, St., *Deutsch. med. Wchnschr.*, 1913, xxxix, 1584.

in paraffin and sectioned as thinly as possible. They were then stained with Giemsa solution and decolorized according to the method of Noguchi.[3]

Short chains of the organism were observed in the tissue which had remained in the incubator for six days. They consisted of three distinct globules about 0.2 of a micron in diameter, not perfectly round, arranged in chain formation, and stood out sharply as purplish red dots against the faint blue of the autolyzed brain tissue (figure 1). They are seen best under artificial light.

By examining sections of tissue removed on succeeding days, a gradual development of the organism could be traced. After eight days' growth (figure 2) five divisions could be seen, and the slight variation in size was increased by the tenth day, when nine divisions still in chain formation were seen (figure 3). The difference in size is accentuated in the photograph on account of the inclination of the plane in which the chain lay.

After twenty days' incubation sixteen bodies were found approximated, describing a loop like a twisted letter U. They did not lie in the same plane and for that reason no photograph was taken. Thirty day sections contained the organism growing in bunches, as shown in figure 4. Here the bodies were slightly smaller but were perfectly distinct.

Similar experiments were carried out successfully with the brains of monkeys infected with K virus. One of the protocols is as follows:

Monkey B.—Inoculated intraspinously with 3 c.c. of paper filtrate of K virus suspension, developed a paralysis in five days, and died two days later. Pieces of the right brain were incubated and treated according to the method already described, and the organism was found in short chains.

The minute microörganisms present in the incubated nervous tissues are clearly identical with those cultivated by Flexner and Noguchi and stained by them in film preparations and sections of freshly prepared tissues. The differences in grouping and size can be accounted for by the conditions of multiplication in the brain tissue removed from the body. That the bodies observed are organisms and not granules of autolyzed cells is clear.

[3] Flexner, S., and Noguchi, H., *loc. cit.*

[4] Flexner, S., and Lewis, P. A., *Jour. Exper. Med.*, 1910, xii, 253.

Since the organisms multiply within the nervous tissue when incubated in this manner, the logical procedure for obtaining cultures would be to allow them to develop and then set them free by some means into the fluid.

Bits of monkey brains known to contain K virus were incubated in the kidney-ascitic fluid culture medium. Examinations by smear preparations of the fluid from time to time up to fifty days gave no evidence that a culture had been obtained. The pieces of nerve tissue were then crushed by means of a glass rod and the tubes incubated for eleven days more, when the organisms were found in pure culture. The organisms grew in subcultures from these tubes.

Incubation for twelve days before crushing the bits of brain, and further incubation for three days before making subcultures is sufficient to obtain good growths.

Flexner and Lewis[4] have shown that the virus of poliomyelitis may occur in the circulating blood, and recent experiments by Clark, Fraser, and Amoss confirm and extend the earlier results obtained by Flexner and Lewis. On the other hand, the indications are that the virus is not present in the blood either abundantly or constantly. An effort was therefore made to detect the microörganism in blood films with a successful result in one instance.

Monkey C. Macacus rhesus.—Inoculated intraspinously with 1 c.c. of a suspension of K virus, ran a typical course, and became prostrate in ten days. The animal was etherized on the twelfth day and film preparations were made from the heart's blood. These were fixed in methyl alcohol and stained for twelve hours in 1:20 Giemsa solution and decolorized by standing in acetone for eight hours. Two short extracellular chains were found, each consisting of three globules identical in morphology and staining properties with the previously described organism. One of these sets is shown in figure 5.

Monkey D. Macacus rhesus.—Received intravenously 180 c.c. of a Berkefeld filtrate of K virus. Eleven days later typical poliomyelitis ensued and the monkey was moribund on the fourteenth day, when it was etherized and 2 c.c. of heart's blood were placed into kidney-ascitic culture medium. Fifteen days later film preparations did not disclose the presence of the organism, but transplants were made and the organism was found in pure culture after twenty days' incubation.

SUMMARY.

The globoid bodies, or minute microörganisms, cultivated from the central nervous organs of human beings and monkeys that have succumbed to poliomyelitis, may be detected in the incubated brain tissues of infected monkeys in forms indicating post-mortem multiplication. Incubating the poliomyelitic tissues in kidney-ascitic fluid culture medium and then crushing them is a more certain method for obtaining cultures of the organism.

Identical bodies have been detected in blood films prepared on the twelfth day of the acute attack, from a paralyzed poliomyelitic monkey inoculated intraspinously.

The same organism has been cultivated from the blood of a monkey that had received intravenously a large dose of a Berkefeld filtrate of poliomyelitic virus.

No other microörganisms were detected either in the sections of the brain or in film preparations of the blood. These observations tend therefore to confirm the etiological relationship between the minute microörganism and epidemic poliomyelitis suggested by the successful cultivation and inoculation experiments reported by Flexner and Noguchi.

EXPLANATION OF PLATE 35.

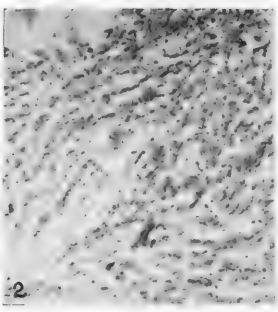
FIG. 1. Globoid bodies in chain formation in brain tissue after six days' incubation. × 1,000.

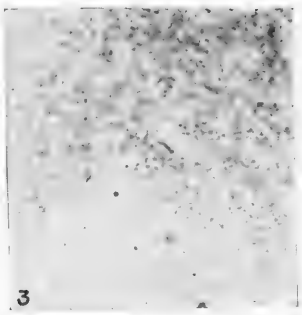
FIG. 2. Globoid bodies in chain formation in brain tissue after eight days' incubation. × 1,000.

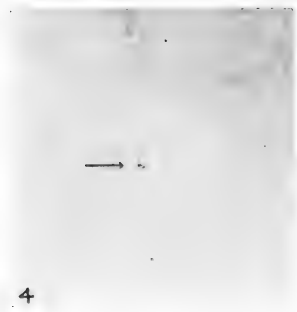
FIG. 3. Globoid bodies in chain formation in brain tissue after ten days' incubation. × 1,000.

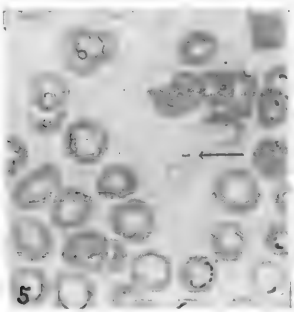
FIG. 4. Globoid bodies in mass formation in brain tissue after thirty days' incubation. × 1,000.

FIG. 5. Globoid bodies in chain formation in heart's blood. × 1,000.









(Amoss: Etiology of Epidemic Poliomyelitis.)

INTRASPINOUS INFECTION IN EXPERIMENTAL POLIOMYELITIS.*

By PAUL F. CLARK, PH.D., AND HAROLD L. AMOSS, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

The routes by which the virus of poliomyelitis may be conveyed to the central nervous organs of monkeys so as to induce infection and paralysis are various but not of equal certainty. Undoubtedly the direct intracerebral injection yields the most constant results, next to which the intrasciatic and intranasal have been placed. Infection by way of the peritoneal cavity, subcutaneous tissues, and blood is obtained with far less certainty. Considerable interest attaches to the intraspinous route of inoculation of the virus, because of the bearing which it may have on the theory of the pathogenesis of poliomyelitis.

That the virus of poliomyelitis exhibits a great affinity for the nervous organs is obvious from the location of the main lesions of the disease. There is, however, lack of agreement as to the manner in which the lesions are produced; namely, whether through direct action of the virus upon the nerve cells or through indirect effect of lesions in the blood vessels and ground substance. The hypothesis that poliomyelitis is a specific affection of the anterior grey matter of the spinal cord has been abandoned. The lesions occur regularly throughout the structures of the cord including the intervertebral ganglia, frequently in the medulla and brain, and quite often in the Gasserian and abdominal sympathetic ganglia.[1] Probably other ganglionic masses, not yet studied, will show effects. Besides, lesions are present in the lymphatic and other somatic organs.[2]

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[1] Flexner, S., Clark, P. F., and Amoss, H. L., *Jour. Exper. Med.*, 1914, xix, 205.

[2] Flexner, S., Peabody, F. W., and Draper, G., *Jour. Am. Med. Assn.*, 1912, lviii, 109.

The pia-arachnoid membranes of the central nervous system are constantly involved in the pathological process, although the degree of involvement varies. Examination of the cerebrospinal fluid in human cases regularly shows an increase in cells and globulin, thus indicating an inflammation of the meninges.[3] Histological examination of the spinal cord of fatal human and experimental cases shows cellular infiltration of the pial membrane, particularly adjacent to the surface of the spinal cord and most marked near the blood vessels of the membrane. The pia projecting into the anterior and in a less degree into the posterior median columns contains this cellular infiltration, which, within the grey substance of the spinal cord, may be strongly marked about the blood vessels and even in the ground substance. While some degree of infiltration is never absent, cases in man and in the monkey occur in which it is slight. A similar infiltration exists in the intervertebral ganglia appearing to extend from the pial investment into their substance. Along with the cellular infiltration are nerve cell degenerations of various grades. Sometimes the infiltrations predominate in degree over these degenerations, sometimes the degenerations predominate over the infiltrations.

Flexner has emphasized the nasopharyngeal mode of infection in poliomyelitis, and according to his view the virus ascends probably by way of the lymphatics from the nasal mucous membrane and multiplies in the pia-arachnoid membranes and the adjacent nervous structures of the brain before becoming established in the medulla and spinal cord.[4] Hence it has seemed important to ascertain whether infection can be readily produced by introducing the virus directly into the subarachnoid spaces. This may be done by a subdural injection into the region of the brain after trephining or into that of the spinal cord by means of lumbar puncture. The latter is the simpler and safer method, as there is little risk of introducing the fluid into the nervous tissues themselves.

Thus far Flexner and Lewis[5] alone seem to have recorded a suc-

[3] Peabody, F. W., and Draper, G., *Am. Jour. Dis. Child.*, 1912, iii, 153.

[4] Flexner, S., *Jour. Am. Med. Assn.*, 1910, lv, 1105; Huxley Lecture, *Lancet*, 1912, ii, 1271, and *Science*, 1912, xxxvi, 685.

[5] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1910, liv, 535.

cessful infection among several failures by means of intraspinous inoculation of the virus, although Neustaedter and Thro caused poliomyelitis in three monkeys by means of combined intraspinous and subcutaneous inoculation,[6] and Römer,[7] without giving any experimental details, remarks that intraspinous infection takes place readily.

No special difficulty surrounds the intraspinous mode of infection. The requirements are an active virus, such as our M A and K strains, and a dose somewhat larger than that necessary for successful intracerebral inoculation. However, the immediate effects of the two modes of injection are different. When the virus is injected into the brain it is usually deposited in a small cavity within the cerebral hemisphere although some may escape into the meninges. It may safely be assumed that multiplication of the virus soon begins under the favorable conditions of temperature and location, and that gradually it passes into the adjacent membranes and general nervous tissue in which further multiplication occurs. When the virus is injected into the subarachnoid spaces in the lumbar region it quickly diffuses in the membranes along the length of the spinal cord and base of the brain, but it begins also immediately to pass with the cerebrospinal fluid into the circulating blood through the usual venous channels. Since the blood route is perhaps the poorest for producing infection the virus which escapes from the subarachnoid spaces into the veins may be regarded as lost.

Hence, instead of Berkefeld filtrates we have found it better to employ paper filtrates of the usual 5 per cent. emulsion of the infected nervous tissue containing the virus. In a series of nine monkeys inoculated by lumbar puncture with paper filtrates of M A virus, all developed poliomyelitis. Of this series six monkeys became paralyzed, while three developed definite symptoms without showing paralysis. The microscopical examination of the spinal cord and intervertebral ganglia of the latter three brought out the presence of typical poliomyelitic lesions of mild degree.

[6] Neustaedter, M., and Thro, W. C., *New York Med. Jour.*, 1911, xciv, 813.
[7] Römer, P. H., Die epidemische Kinderlähmung, Berlin, 1911, 132.

ILLUSTRATIVE PROTOCOLS.

Experiment 1. Macacus rhesus.—Feb. 18, 1913. A small quantity of cerebrospinal fluid was withdrawn by lumbar puncture and 1 c.c. of a paper filtrate from a 5 per cent. suspension of the spinal cord from a recently paralyzed M A monkey was injected. Feb. 22. Tremor; left arm paralyzed; extremities weak. Feb. 23. Paralysis of arms, back, neck, and right leg. Feb. 24. Prostrate. Etherized. Characteristic lesions of experimental poliomyelitis present in the spinal cord and intervertebral ganglia.

Experiment 2. Macacus rhesus.—Feb. 26. 0.5 c.c. of cerebrospinal fluid was withdrawn by lumbar puncture, and 0.5 c.c. of a paper filtrate prepared from a recently paralyzed animal was injected. Mar. 3. Weakness of left arm. Mar. 7. Extremities paralyzed; prostrate. Mar. 8. Etherized. Lesions of poliomyelitis present.

Experiment 3. Macacus rhesus.—May 22. Cerebrospinal fluid was withdrawn by lumbar puncture, and 1.2 c.c. of a paper filtrate prepared from a recently paralyzed animal was injected. May 29. Excitable; tremor. May 30. Legs weak. May 31. Legs and arms weak. June 2. Excitability increased; neck weak; weakness of arms and legs increased. June 7. Condition stationary. Etherized. The spinal cord and intervertebral ganglia showed typical but mild lesions of poliomyelitis.

The protocols given serve as examples of the remaining experiments. From them it can be concluded that given a specimen of virus of adequate virulence experimental poliomyelitis can be regularly produced by intraspinous inoculation.

The virus having been introduced into the subarachnoid spaces clearly gains access to the nervous tissues of the spinal cord and medulla, with the interstices of which the cerebrospinal fluid is in intimate communication. A part, therefore, becomes fixed to the tissues within the pial membrane and about the blood vessels and doubtless to the nervous structures proper. Another part readily leaves the subarachnoid spaces with the cerebrospinal fluid. Thus far no one has detected the virus in the cerebrospinal fluid in human cases of poliomyelitis and it is usually absent from the fluid in monkeys at the time of the onset of paralysis, although it may be present at an earlier period after intracerebral inoculation.[8] We have, therefore, tested the cerebrospinal fluid at intervals of twenty-four and forty-eight hours after the inoculation, and at the expiration of six days when the first symptoms of paralysis made their appearance.

[8] Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1911, lvii, 1685.

Macacus rhesus A.—Nov. 22. Intraspinous injection of 3 c.c. of a paper filtrate of K virus was given after withdrawal of 2 c.c. of clear cerebrospinal fluid. Nov. 23. 0.8 c.c. of turbid fluid was withdrawn by lumbar puncture. Nov. 24. 1 c.c. of less turbid fluid. The fluids were inoculated intracerebrally into rhesus monkeys. Nov. 26. Excitable; legs paralyzed; back weak. Nov. 27. Prostrate. Died. Typical poliomyelitic lesions present in the spinal cord.

Macacus rhesus B.—Nov. 13. Intracerebral injection made of the 24 hour specimen of fluid withdrawn by lumbar puncture from monkey A.

Nov. 30. Excitable. Dec. 2. Paralysis of left leg. Dec. 4. Muscles of extremities and back paralyzed; prostrate. Dec. 5. Etherized. Lesions of poliomyelitis present.

Macacus rhesus C.—Nov. 24. Intracerebral injection of 1 c.c. of spinal fluid withdrawn by lumbar puncture from monkey A 48 hours after intraspinous inoculation.

Nov. 29. Excitable; right arm and leg paralyzed; back weak. Nov. 30. Prostrate. Dec. 2. Etherized. Lesions of poliomyelitis present.

Macacus rhesus D.—Nov. 22. 1 c.c. of a paper filtrate of the virus was given by intraspinous injection. Nov. 28. First symptom of infection. 2 c.c. of cerebrospinal fluid were withdrawn, and on Nov. 29 injected intracerebrally into another rhesus monkey, E. No symptoms developed in the latter.

These experiments show again, first, that infection by way of the subarachnoid spaces readily occurs, and next, that the virus even when introduced directly into them tends to escape from these spaces partly into the central nervous tissues, where it is held and in which it multiplies, and into the general circulation by which it is probably carried to all the nervous organs which doubtless again in some degree remove it from the blood. The reasons for failure to detect the virus in the cerebrospinal fluid in human cases are now apparent.

SUMMARY.

By intraspinous injections of specimens of poliomyelitic virus of suitable virulence infection can be caused regularly in *Macacus rhesus* monkeys.

The virus passes from the subarachnoid spaces into the nervous tissues in which it multiplies, and into the blood.

The constant involvement of the pia-arachnoid membranes in poliomyelitis, even when no paralysis occurs, and the fact that infection can readily be produced by intraspinous inoculation suggests anew that in the pathogenesis of poliomyelitis the interstitial tissue changes within the meninges, blood vessels, and ground substance play a determining part.

While the virus injected into the subarachnoid spaces can be demonstrated there by inoculation tests forty-eight hours after the injection it can no longer be detected on the sixth day, at a time when the first symptoms of infection make their appearance. The failure of the cerebrospinal fluid from human and experimental cases of poliomyelitis to produce the disease when inoculated into monkeys is due to the fact that the virus is either fixed by the nervous tissues or passes into the blood.